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# STUDIES OF THE RAW EGG WHITE SYNDROME IN RATS<sup>1</sup>

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ONE FIGURE

(Received for publication November 9, 1933)

A novel syndrome occurring in rats which received Chinese dried egg white as the dietary source of protein was reported by Boas ('27). The chief symptoms were a characteristic 'eczematous dermatitis,' accompanied by alopecia, blepharitis, spasticity, and in some cases by edema of the feet. Skin hemorrhages were seen in severe cases. The similarity of the symptoms to those of pellagra was recognized. The condition was prevented by inclusion of one of the following substances in the diet: raw potato starch, arrowroot starch, dried yeast, fresh egg white, egg yolk, milk, commercial casein, crude lactalbumen, spinach, cabbage, banana, or dried horse serum. The cereal starches, wheat bran, marmite, malt extract, butter, or raw meat failed to prevent the occurrence of the symptoms. The syndrome did not develop, however, if the egg white was cooked before drying. In a later paper (Fixsen, '31) the same author<sup>2</sup> presented data to support the hypothesis that a toxic substance formed during the drying process caused the disease. It was said that ovalbumen, ovoglobulin, or the total albumen and ovomucoid fraction prepared from crude egg white furnished satisfactory protein for young rats and that the nutritive properties of these fractions were not altered by desiccation as was the case with the crude egg

<sup>1</sup>Published with the permission of the director of the Alabama Experiment Station.

<sup>2</sup>Mrs. Boas became Mrs. Fixsen.



white. Moreover, it was possible to prepare, from dried egg white, fractions which were non-toxic, by precipitation with ammonium sulfate.

The earlier results of Boas were confirmed by Findlay and Stern ('29) who reported that raw egg white was protective and that raw liver had a high protective potency. These investigators suggested that the egg white syndrome in rats is the analogue of pink disease (Swift's disease, erythredema) in children.

Parsons ('31) has studied the effects of diets containing 20 to 66 per cent of egg white. She has observed, in addition to the symptoms observed by Boas, a high rate of mortality in young rats soon after they were placed on the egg white diets. This was largely prevented by the feeding of liver either before or after the rats were weaned.

In three papers which appeared after the completion of the experiments reported in the following pages, Parsons and co-workers have discussed additional findings. They (Parsons and Kelly, '33) have concluded: A) that the tendency to produce a pellagra-like condition in rats is a property of native egg white rather than a result of desiccation; B) that the injury involves an interrelation between a positive toxicity and a relative absence of a protective factor; and, C) that the toxicity of the native egg white is destroyed by heat. They also found ('33) that the injurious property of the egg white was destroyed by incubation with acid pepsin or mild hydrochloric acid alone but persisted in egg white denatured with alcohol. The protein precipitated by complete saturation of egg white solution with ammonium sulfate was found to be toxic. In addition, it was observed (Parsons, Lease and Kelly, '33) that dried yeast, dried egg yolk, wheat embryo and dried milk were only moderately protective, a concentration of one to three times that of the 'toxic' egg white in the diet being necessary for protection. Cooked beef liver, pork liver, and beef kidney equivalent to one-fourth of the egg white in the diet were curative. Spleen, heart, ovaries, adrenals, blood, hemoglobin, or liver extract no. 343 (Lilly) had little or no

potency. The activity of liver, which was said not to depend on its nucleo-protein fraction, was decreased by boiling for 1 hour with 5 per cent HCl and destroyed by heating cooked liver 6 days at 100°.

Ringrose, Norris and Heuser ('31) have reported "lesions of a distinctly pellagrous character" in chicks which received diets containing dried egg albumen.

On the other hand, Chick, Copping and Roscoe ('30) have found that 2.5 to 5.0 gm. of fresh, cooked egg white (dry weight 0.3 to 0.6 gm.) cured the skin lesions and restored 'normal' growth in rats receiving a diet deficient in vitamin G (B<sub>2</sub>). The rate of growth was not maintained, however, when egg white was the sole source of vitamin G.

The similarity of the symptoms of the egg white disease to the symptoms produced by Salmon ('31) in rats receiving diets in which the casein and the yeast had been subjected to dry heat treatment led to a study of the egg white syndrome in this laboratory. The results of the study are reported in the following pages.

#### EXPERIMENTAL METHODS

*Animals and their care.* A mixed strain of albino-hooded rats was used. In preliminary experiments with egg white diets it was found that, when young rats were transferred to the experimental diets at weaning time and given food ad libitum, a high rate of mortality resulted. This was caused by an acute hemorrhagic nephritis which usually developed in the period from the tenth to the fifteenth day of the experiment. It was apparently the condition which has been referred to by Parsons as the 'initial injury' resulting from egg white diets.

For the experiments reported in this paper a special routine was developed which largely avoided this early injury. After the young were 17 days old, the dam was removed from the maternity cage each morning about 7 to 8 o'clock and placed in a separate feeding cage where she had access to the stock diet. At the same time the experimental diet was placed in

the cage with the young. The dam was returned to the maternity cage about 10 to 11 o'clock, left with the young for approximately 3 hours, and was again removed to the feeding cage until late afternoon when she was returned to the maternity cage for the night. The experimental diet was always removed when the dam was with the young. The young were weaned at 27 days of age and placed on experiment. They were started on 3 gm. of the experimental diet per rat daily. The diet was slowly increased until at the end of 2 weeks it was being supplied *ad libitum*. Fresh portions of the diets were placed in the feed jars and distilled water was supplied in clean vessels each day. Individual cages with raised screen bottoms were used in all of the experiments.

*Experimental diets.* The composition of the experimental diets is shown in table 1. The raw, dried egg white was a Chinese product in the form of large yellow flakes, 'crystals.' It was completely soluble.

The cooked, dried egg-white was prepared by adding 3 liters of  $H_2O$  to 2 kilos of the dried egg white and leaving the mixture in the refrigerator until the material was completely dissolved. The solution was then heated in an electric oven for 30 minutes at 90 to 100°C. and the coagulated mass was spread in a thin layer and dried at 37°C.

The raw, fresh egg white was from fresh eggs obtained from the station poultry farm. The eggs were carefully broken and the whites separated from the yolks each day just before feeding.

The extracted egg white was prepared by pouring 11 liters of fresh egg white into 20 liters of boiling  $H_2O$  containing 50 ml. of concentrated HCl. The mixture was again brought to the boiling point, the coagulum was removed and, after the addition of 50 ml. concentrated HCl, another 11-liter batch of egg white was poured into the boiling extract. The extract was then drawn off. The two batches of coagulated albumen were combined and extracted by percolating with 51 per cent alcohol<sup>3</sup> until the extract was nearly colorless. A total of

<sup>3</sup> The strength of alcohol is expressed in this paper as per cent by weight.

TABLE 1  
*Composition of diets*

Diet	A	A-1	B	B-1	B-2	C	C-1	C-2	C-3	C-4	D	E	F	G	H	I	J
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Raw dried egg white	18.0	18.0	66.0	66.0	66.0	18.0	18.0	18.0	18.0	18.0	18.0	12.0			150.0 <sup>a</sup>	18.0	18.0
Raw fresh egg white																	
Cooked dried egg white																	
Extracted egg white																	
Extracted casein																	
Yeast residue	10.0	10.0	10.0	10.0	20.0	10.0	10.0		10.0	18.0			12.0			18.0	18.0
Hydrolyzed yeast residue																	
Ground white corn																	
Sucrose	77.0	67.0	29.0	19.0	9.0	56.5	46.5	46.5	46.5	38.5	56.5	85.0	85.0	56.5	56.5	56.5	56.5
Salt mixture 186 <sup>a</sup>	4.0	4.0	4.0	4.0	4.0	4.5	4.5	4.5	4.5	4.5	4.5			4.5	4.5	4.5	4.5
Agar	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0			1.0	1.0	1.0	1.0
Filtered butterfat																	
Cod liver oil <sup>b</sup>						18.0	18.0	18.0	18.0	18.0	2.0			18.0	18.0	18.0	18.0
Hydrogenated cottonseed oil						2.0	2.0	2.0	2.0	2.0	2.0			2.0	2.0	2.0	2.0
Calcium carbonate											18.0	2.0	2.0				
Sodium chloride												1.0	1.0				

<sup>1</sup> J. Biol. Chem., 1927, lxxiii, 487.<sup>a</sup> When cod liver oil was not incorporated in the basal diet, each rat received 0.10 ml. per day.<sup>b</sup> The fresh egg white was added to each rat's weighed portion of the mixture of other constituents daily.

133 liters of extract, including the first aqueous liquor, was obtained. The coagulum was pressed as dry as possible in a hand press and placed in current of air from fan at 37°C. until nearly dry. The drying was completed in a forced-draft oven at 70°C. and the dried product was then ground in a burr mill. A total of 2060 gm. of the dry extracted material was obtained from the 22 liters (22.83 kilos) of fresh egg white.

The egg white extract was prepared by concentrating<sup>3</sup> the 133 liters of extract obtained from the coagulated egg white. When the volume was reduced to 12 liters the concentrated extract was cooled, shaken with ether, and the aqueous layer filtered through a fluted filter. The filtrate was then concentrated until 1 ml. was equivalent to 2 gm. of egg white (dry basis).

The brewer's yeast was Vita-Food No. 1, Red Label, pasteurized brewer's yeast. The baker's yeast was Northwestern dehydrated yeast.

The yeast residue was prepared from Vita-Food No. 1, Red Label, unpasteurized brewer's yeast. Forty-five kilos of this yeast were placed in an enamel-lined kettle and percolated with 51 per cent alcohol until a total of 500 liters of extract was obtained. Fifteen liters of 93 per cent alcohol were then poured over the extracted yeast to facilitate the dehydration and the material was sucked as dry as possible by vacuum. The extracted residue was then spread in a thin layer and dried in a current of air at 35 to 40°C.

The hydrolyzed yeast residue was the product resulting from adding 2.5 liters of a solution containing 100 ml. of concentrated HCl per liter to 500 gm. of the extracted residue described above and boiling on an asbestos bath for 5 hours. The excess HCl was neutralized with NaOH leaving the preparation just slightly acid to litmus. The preparation was then dried in a forced-draft oven at 70°C.

Extract B-G was obtained by concentration<sup>4</sup> of the first 400 liters of extract from 45 kilos of brewer's yeast until 1 ml.

<sup>4</sup> The concentration of extracts was always done under diminished pressure.

of the extract was approximately equivalent to 2 gm. of the original yeast. The concentrated extract was then shaken with ether to remove fatty material and after standing in refrigerator 2 to 3 weeks was filtered while cold through fluted filters. The volume was adjusted to make 1 ml. equivalent to 2 gm. of yeast.

Extract B was prepared by treating a yeast extract similar to the above with 1 gm. of English fuller's earth for each 23 to 30 gm. of yeast,<sup>5</sup> the extract having previously been adjusted to about pH 3.50. The adsorbate was filtered out, thoroughly washed with  $H_2O$ , dried, and the vitamin B extracted from the dried solid with pyridine-acetate in 80 per cent alcohol (Salmon, '31). The extract was concentrated and subjected to fractionation by increasing strengths of alcohol until a fraction soluble in absolute alcohol was obtained. The vitamin was then precipitated from aqueous solution by tannic acid, the precipitate dissolved in acetic acid, and the tannic acid removed with Pb acetate. The filtrate was concentrated to near dryness, the residue extracted with  $H_2O$ , and the solution dealed with  $H_2SO_4$ . The filtrate was made to volume with 25 per cent alcohol and its vitamin B potency determined by testing on pigeons. This extract furnished a potent source of vitamin B but was free from vitamin G.

The dried liver was prepared by drying ground beef liver in a forced-draft oven at 70°C.

The liver residue was prepared by extracting fresh, ground beef liver with 51 per cent alcohol, allowance being made for the moisture in the fresh material. Extraction was continued until 65 liters of extract were obtained. The extracted residue was dried at 70°C.

The liver extract was obtained by concentrating the above 65 liters of extract to 3 liters, shaking with ether to remove fatty material, and concentrating the aqueous layer to 850 ml. After standing in a refrigerator for 10 days, the extract was

<sup>5</sup> The amount of fuller's earth used depended upon the potency of the yeast as determined by protective tests with pigeons.

filtered and the volume adjusted so that 1 ml. corresponded to 1 gm. of the material in the extracted residue.

The casein was extracted by percolating with water acidulated to 0.20 per cent with acetic acid for 1 week and then with 20 liters of 93 per cent alcohol per 45-kilo batch of casein to facilitate drying. The extracted casein was then sucked as dry as possible by evacuation and dried in current of air at 35 to 40°C. It was ground to pass through a 40-mesh sieve before being mixed into the basal diet. Tests showed this material to be free from vitamins B and G.

*Duration of experiments.* The length of the experimental period was 14 weeks, except in some cases where the animals died, or where they were used for curative tests, or where the outcome was obvious in a shorter period.

## RESULTS

*Basic raw egg white diets.* Table 2 shows the weights of rats which received the basic raw egg white diets. Group 1 received diet A containing 18 per cent of raw, dried egg white and group 3 received diet B containing 66 per cent of raw, dried egg white. Both groups received sufficient extract B to furnish vitamin B in excess of the normal requirement. (Preliminary experiments confirmed the findings of Chick and co-workers ('29, '30) that egg white is lacking in vitamin B.) The behavior of the two groups was very similar. A rigid limitation of the food intake during the first week of the test was necessary to control diarrhea; as a result there was some loss in weight. Despite the gradual increase of food to ad libitum feeding by the beginning of the third week, the rats were unable to make appreciable gains on either diet. When extract B-G was substituted for extract B, the rate of growth was increased on diet A (group 2).

Since diet A was a low fat diet and was probably deficient in the unsaturated fatty acids which have been shown (McAmis et al., '29; Burr et al., '29, '30, '32) to be essential for normal nutrition, the effect of adding 0.20 ml. of linseed oil per rat daily was determined. This addition increased

the rate of growth significantly when extract B was fed (group 4) but increased it even more when extract B-G was fed (group 5).

The rate of growth on the medium fat diets C and D (groups 6 and 9) was comparable to that on diet A supplemented with linseed oil. Again the substitution of extract B-G for extract B produced a favorable response on the growth rate (groups 7 and 10). Diet C was not improved by the further addition of linseed oil (group 8). Growth was slightly improved by the addition of linseed oil to diet D (group 11) but severe skin lesions developed and an attendant decline in weight occurred earlier in the group which received the linseed oil; this was possibly associated with a slightly

TABLE 2

*Average weekly weights of rats. Basic raw dried egg white diets*

Group	1	2	3	4 <sup>a</sup>	5 <sup>a</sup>	6	7	8 <sup>a</sup>	9	10	11 <sup>a</sup>
Diet	A	A	B	A	A	C	C	C	D	D	D
Extract	B <sup>1</sup>	B-G <sup>2</sup>	B <sup>1</sup>	B <sup>1</sup>	B-G <sup>2</sup>	B <sup>1</sup>	B-G <sup>2</sup>	B-G <sup>2</sup>	B <sup>1</sup>	B-G <sup>2</sup>	B-G <sup>2</sup>
Number of rats	4	4	4	2	4	5	8	4	4	4	4
<i>Week</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Initial	48	48	50	53	47	47	53	49	51	52	52
1	42	41	44	67	43	50	55	59	59	66	64
2	49	50	53	82	52	58	72	77	62	74	84
3	54	67	59	91	71	68 <sup>a</sup>	91	94	74	83	102
4	58	80	69	96	87	76	110	109	81	99	114
5	62	91 <sup>a</sup>	74	106	101	83	125	123	85	112	128
6	57 <sup>a</sup>	91	70	108	106	88	139	123	89	122	135
7	66	100	70	113	118	88	145	128	94	128	139
8	61 <sup>a</sup>	105	66	106	129	107 <sup>a</sup>	146	134	90	131	132
9	68 <sup>a</sup>	109	65	99	139	110	143	128	94	132	122
10	79	110	58	99	145	109	144	125	95	133	118
11	70	110	60 <sup>a</sup>	97	151	104	141		97	133	119
12	75	110	58		155	115	140		100	133	116
13	75	100	63		158	122	139 <sup>a</sup>				
14	70 <sup>a</sup>	100 <sup>b</sup>			152	123					

<sup>1</sup>In all cases where extract B-G was not given, sufficient extract B was fed to insure an adequate and uniform amount of vitamin B for each rat.

<sup>2</sup>When extract B-G was given each rat received a daily allowance representing the extract from 0.50 gm. brewer's yeast.

<sup>a</sup>Each rat in group 4, 5, 8 and 11 received 0.20 ml. of raw linseed oil daily.

<sup>a</sup>Indicates death of a rat.

<sup>b</sup>Indicates death of two rats.



larger food consumption and higher growth rate in the early part of the experiment. It should be borne in mind that the effect of correcting a deficiency in these diets may be apparent only in the early part of the experiment. Regardless of the correction of minor faults in the diets, the animals eventually decline.

*Symptomatology.* The major features of the symptomatology observed were similar to those described by Boas and by Parsons. In preliminary experiments a high rate of mortality occurred in some groups within the first 10 to 15 days of the experiment. Rats which were changed suddenly to diets containing as little as 18 per cent of raw, dried egg white and given food ad libitum usually showed no abnormal symptoms for 8 to 10 days, with the exception of some diarrhea. Then individual rats would suddenly refuse a large part or all of the day's allowance of food. These animals appeared extremely nervous and excitable for a few hours but gradually became lethargic and evidenced muscular weakness, continuous tremor, excess lacrimation, and incontinence of urine. These symptoms developed rapidly and rats became comatose and died, frequently within 6 to 8 hours after the first symptoms were observed. At necropsy<sup>6</sup> an acute hemorrhagic nephritis was invariably found.

In the series of experiments reported in this paper, this acute injury was avoided to a large extent by the method of feeding. A few rats showed mild symptoms but appeared to recover; in such cases evidence of old injury was apparent in the kidneys at necropsy after the completion of the experiment. Old lesions were also found in the kidneys of some animals which had never shown symptoms of injury.

With the exception of some diarrhea, the usual history in these experiments was uneventful during the first 4 or 5 weeks. On the basic diets supplemented with extract B there was very little growth; when these diets were supplemented with extract B-G considerable growth occurred. Following this there

<sup>6</sup> The authors express their appreciation to Mr. C. O. Prickett for the necropsy reports.

gradually developed the pigmentation and scaliness of the skin, the peculiar matted or ribbed appearance of the hair, alopecia, fissuring of the oral commissures, blepharitis, a variable edema and erythema of the feet, and evidence of nervous derangement as described by the above investigators. In addition, there has been rather constant occurrence of a profuse salivation, ulceration of the tongue, and a grayish spongy appearance of the buccal mucosa, in the later stages of the disease.

The skin lesions have been more severe and slightly different in character on rats receiving diets A and B than on those receiving diets C and D, or diets A and B supplemented with linseed oil. The rats on the low fat diets began to develop dry, rough skins about the third or fourth week. The epithelium exfoliated in small white scales which at first remained on the skin or were retained by the short, fur-like hair. Later there was desquamation of the epithelium in larger, thicker scales which would bring away tufts of hair. Deep fissures often appeared in the skin and from these blood exuded to form heavy brown incrustations. The scaly condition usually involved the feet, tail, and ears. The addition of linseed oil to diets A and B or the presence of 18 per cent of fat in other diets, had a pronounced effect in reducing the scaliness and fissuring of the skin. Instead of a dry condition, there was an excessive seborrheic secretion and a brown pigmented coating formed on the surface of the skin, particularly in the sacral and lumbar regions, when the fats were added to the diet; it was only in the last stages of the disease that any marked exfoliation of the epithelium occurred, the skin frequently remaining free from scaliness even after there was practically complete depilation. Some of the animals, however, produced severe traumatic lesions by biting or by lacerating the skin with the claws. The surface hemorrhages which occurred on the rats receiving the low fat diets were rarely seen on those receiving the medium fat diets; however, hemorrhages within the skin, which appeared as large brown pigmented areas, were not infrequent. On both types of diet

there usually appeared to be a congestion of the superficial blood vessels which gave the skin a peculiar pink color even in the early part of the experiment.

*Gastric acidity and hemoglobin.* It was frequently noted at necropsy that the stomach contents of rats showing a severe form of the egg white syndrome gave a negative Congo red test. Gastric samples were, therefore, obtained from a series of fifty rats which were receiving various egg white diets. The samples were taken following a 24-hour fast. Five milliliters of 1 per cent alcohol were then administered to each rat and 30 minutes later 3 ml. of  $H_2O$  were administered and as much as possible of the stomach contents aspirated immediately. The H-ion concentration of the specimens was determined by the quinhydrone method.

For rats not showing marked symptoms the gastric samples were usually within the range of pH 2.20 to 3.00. In the late stages of the disease, however, there was in most cases a significant decrease in the H-ion concentration, the range being from pH 4.00 to 6.50.

Hemoglobin determinations were made on the blood from sixty rats in various stages of the disease. Of these, twenty-three were well below normal values, being within the range of 10 to 12.5 gm. per 100 cc. There was apparently a tendency for the hemoglobin to decrease in the late stages of the disease. This tendency was less apparent on the low fat than on the medium fat diets.

*Yeast and liver as supplements to the egg white diets.* Table 3 shows the weights of rats which received dried brewer's yeast, dried baker's yeast, dried liver, liver extract, and the extracted residue of liver as supplements to basic egg white diets A and C.

The rate of gain was markedly increased and the occurrence of symptoms of the egg white disease was prevented by the feeding of 0.50 gm. of brewer's yeast per rat daily. The gains were much greater on diet C (group 13) than on diet A (group 12). The rats on diet A showed a dry, rough condition of the skin which was probably a mild form of the unsaturated fatty

acid deficiency disease. This was very mild, however, and was not to be compared to the miserable condition of the skin in groups 1, 2 and 3.

Baker's yeast (group 14) was much less effective than brewer's yeast, 0.50 gm. being little more effective in increasing the gain than a corresponding amount of extract B-G.

TABLE 3

*Average weekly weights of rats. Raw dried egg white diets supplemented with brewer's yeast, baker's yeast, dried liver, liver residue, or liver extract*

Group	12	13	14	15	16	17
Diet	A	C	C	C	C	C
Extract	—	—	—	B	B	B
Supplement <sup>1</sup>	Brewer's yeast	Brewer's yeast	Baker's yeast	Dried liver	Liver residue	Liver extract
Number of rats	6	4	6	4	4	4
Week	gm.	gm.	gm.	gm.	gm.	gm.
Initial	48	53	51	53	54	54
1	48	80	51	72	74	69
2	68	108	71	96	91	79
3	92	135	92	107	106	88
4	113	154	108	107	116	92
5	130	176	119	124	127	102
6	144	193	127	136	134	115
7	157	211	137	148	142	128
8	168	223	145	158	151	134
9	172	234	149	167	158	141
10	180	248	156	174	165	146
11	188	264	158	178	168	148
12	—	—	162	182	173	150
13	—	—	—	189	176	155
14	—	—	—	194	181	160

<sup>1</sup> All supplements fed at rate of 0.50 gm. per rat daily except the liver extract which was fed at rate equivalent to 0.50 gm. of the extracted residue.

The rats receiving the baker's yeast developed the usual symptoms although probably in slightly less severe form.

Dried liver (group 15) was less effective than brewer's yeast in increasing the rate of gain but prevented all symptoms of the egg white disease. Liver residue (group 16) was only slightly less effective in promoting growth than the whole

dried liver, 0.50 gm. per rat daily preventing the occurrence of abnormal symptoms. Liver extract (group 17) equivalent to 0.50 gm. of the residue per rat daily was inferior to dried liver or liver residue in its effect on growth as well as in preventing the occurrence of abnormal symptoms. It had some protective value as the rats receiving the extract did not develop pronounced skin lesions. They did show, however, the short fur-like hair which was always seen as an early symptom.

*Egg white + yeast residue and egg white + casein diets.* The effect of adding yeast residue, hydrolyzed yeast residue, or extracted casein to the basic raw egg white diets is indicated by the weights of rats in table 4. The addition of 10 per cent of yeast residue to diet A produced a tremendous increase in the rate of gain (group 18). The yeast residue had an even more striking effect on the condition of the rats, group 18 appearing normal in every way except for a slight dry, roughened condition of the skin which was prevented by the addition of 0.20 ml. of linseed oil per rat daily (group 20).

The addition of 10 per cent of yeast residue to diet B, however, did not improve the rate of growth (group 22) nor did it prevent the occurrence of a severe form of the disease. The further addition of linseed oil improved the growth rate slightly (group 23) and effected a striking improvement in the condition of the skin. The addition of 20 per cent of yeast residue likewise failed to produce satisfactory growth (group 24) but the addition of linseed oil increased the growth rate (group 25); growth was still further improved by substituting extract B-G for extract B (group 26). Even in group 26 growth was very poor, although, with the exception of the subnormal rate of growth and a slight seborrheic condition of the skin, there were no apparent abnormal symptoms.

A comparison of group 27 with group 6 shows the response to the addition of 10 per cent of yeast residue to diet C. Growth was further improved by the substitution of extract B-G for extract B (group 28); group 28 made the best growth obtained in this series of experiments.

TABLE 4

*Average weekly weights of rats. Raw egg white diets supplemented with yeast residue, hydrolyzed yeast residue, extracted casein, or white corn*

Group	18	19	20 <sup>1</sup>	21 <sup>1</sup>	22	23 <sup>1</sup>	24	25 <sup>1</sup>	26 <sup>1</sup>	27	28	29	30	31	32	33	34 <sup>a</sup>
Diet	A-1	A-1	A-1	A-1	B-1	B-1	B-2	B-2	B-2	C-1	C-1	C-2	C-3	C-3	C-4	E	F
Extract	B	B-G	B	B-G	B	B	B	B	B-G	B	B-G	B-G	B	B-G	B-G	—	—
Number of rats	2	6	2	5	4	4	4	4	4	4	4	4	4	4	4	6	6
Week	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Initial	53	54	53	55	49	51	51	51	50	49	51	48	52	52	51	52	53
1	61	62	65	64	41 <sup>a</sup>	45	51	56	59	52	81	50	53	78	62	60	65
2	81	87	89	88	47	57	53	63	68	62	111	69	56	102	68	72	83
3	107	113	112	112	54	65	58	68	79	71	138	73	66	121	118	83	98
4	126	138	130	133	60	75	59	73	87	85	160	88	79	136	137	93	119
5	141	158	153	152	63	79	63	80	94	108	183	100	83	149	150	101	141
6	149	177	166	169	60	79	64	81	98	138	210	108	88	144	159	112	160
7	160	182	179	184	61	79	66	88	106	158	230	121	90	139	164	123	170
8	165	200	190	197	69 <sup>a</sup>	79	66	90	115	180	248	141	99 <sup>a</sup>	135	172	133	184
9	182	210	196	208	68	82	67	93	119	197	262	153	102	131	183	144	196
10	191	222	216	221	64	85	65	97	126	204	281	161	102	—	184	150	200
11	199	231	222	231	63 <sup>a</sup>	84	71	103	134	200	286	159	104	—	188	160	218
12	210	241	241	239	63	89	72	106	142	233	292	—	104	—	177	170	228
13	216	241	251	247	61	86	75	109	144	240	296	—	—	—	—	176	236
14	222	243	252	249	—	—	75	111	144	248	304	—	—	—	—	—	—

<sup>1</sup> Each rat in groups 20, 21, 23, 25 and 26 received 0.20 ml. of linseed oil per day.

<sup>a</sup> Group 34, on diet F, is included for comparison with group 33 on diet E.

<sup>a</sup> Indicates death of a rat.

Hydrolysis of the yeast residue largely destroyed its ability to increase growth and prevent the occurrence of symptoms (group 29).

Extracted casein at the 10 per cent level neither improved growth (groups 30 and 31) nor decreased the severity of the usual symptoms; at the 18 per cent level, there was some improvement in the growth of the rats but the syndrome still developed in severe form.

Group 33 received a diet containing 85 per cent of white corn and only 12 per cent of raw, dried egg white. Growth was poor and the usual symptoms developed. Group 34 received the same diet as group 33 except that extracted casein was substituted for the raw, dried egg white; although growth was subnormal in group 34, the rats were in good condition throughout the experiment.

*Effect of various treatments of egg white.* Cooking the dissolved, dried egg white for 30 minutes at 90 to 100°C. increased the rate of gain slightly (group 35, table 5) and decreased the severity of the symptoms; even so, the rats in group 35 were distinctly abnormal.

Raw, fresh egg white (group 36) was not superior to raw, dried egg white. In fact, symptoms developed earlier and appeared to be more severe when the raw, fresh form was fed.

Groups 37 and 38 received diet G containing fresh egg white which had been coagulated by heat and extracted with 51 per cent alcohol. For group 37 the diet was supplemented with extract B, providing a diet which was free from vitamin G. Consequently, these rats made practically no growth. They developed a mild conjunctivitis, ulceration of the tongue, and edema, erythema, and an exfoliating dermatitis of the feet; most of these rats showed a seborrheic condition of the skin, particularly in the sacral region, and a few developed a moderate amount of alopecia. For group 38 the diet was supplemented with extract B-G. The growth of these rats presents a striking contrast to the growth of those receiving a comparable diet containing either raw, fresh egg white







(group 36), raw, dried egg white (group 7), or cooked, dried egg white (group 35). The general condition of the animals in the various groups showed an even greater contrast, group 38 appearing normal in every way except the rate of growth which was below normal. Two of the rats in group 38 received 0.50 gm. of yeast residue per rat daily from the ninth

TABLE 5

*Average weekly weights of rats. Diets containing cooked dried egg white, fresh raw egg white, or fresh egg white coagulated by heat and extracted; also egg white extract as source of vitamin G*

Group	35	36	37	38	39
Diet	G	H	I	I	J
Extract	B	B	B	B-G	B <sup>1</sup>
Number of rats	4	5	6	6	4
Week	gm.	gm.	gm.	gm.	gm.
Initial	50	63	51	52	50
1	50	81	56	73	54
2	59	80	56	89	70
3	64	92	57	107	69 <sup>2</sup>
4	76	97	56	128	70
5	86	100	56	140	78
6	100	100	58	153	89
7	111	101	60	164	97
8	114		57	169	107
9	122		58	178	118
10	126		58	187	125
11	120		60	197	136
12	129		57	202	139 <sup>2</sup>
13	134		57	209	160
14	136		53 <sup>3</sup>	216	185

<sup>1</sup> Group 39 received egg white extract equivalent to 0.60 gm. egg white (dry basis) until the end of the fifth week when it was increased to 2.00 gm.

<sup>2</sup> At the end of the twelfth week extract B-G (equivalent to 0.50 gm. brewer's yeast) was given in addition to the egg white extract.

<sup>3</sup> Indicates death of a rat.

to the fourteenth week of the experiment. There was no evidence that this addition to the diet was beneficial.

*Curative tests.* A number of rats receiving diet C or H supplemented with extract B were used in curative tests. Rapid improvement resulted from the addition of 0.50 gm. of dried liver, brewer's yeast, yeast residue, or 5 to 10 ml.

of whole milk per rat daily. In most cases apparent cures resulted even though the rats were in miserable condition when the supplementary feeding was instituted. No improvement in condition followed the feeding of 0.50 gm. of extracted casein, gelatin, or of extract B-G equivalent to 0.50 gm. of brewer's yeast. Likewise, no response was obtained to the feeding of 0.20 ml. of dilute hydrochloric acid (1:10) per rat daily.



Fig. 1 Rat no. 4233, group 36, receiving diet H which contained raw, fresh egg white.

#### DISCUSSION

The data presented in this paper supplement the work of other investigators in showing that a peculiar physiological abnormality results when rats receive diets containing raw egg white as the sole source of protein. Such abnormality is not dependent upon an extremely high concentration of protein; it is readily produced on diets containing only 18 per cent (dry basis) of raw egg white. Nor is it necessarily associated with the use of egg white in the dried form; diets containing raw, fresh egg white produce just as disastrous results as those containing the dried material.





The most prominent symptoms produced by such diets are short, fur-like or woolly hair, alopecia, hyperemia, exfoliating dermatitis, skin hemorrhages, variable edema, and erythema with desquamation of the epithelium of the feet, conjunctivitis and blepharitis, salivation, ulceration of the tongue, spongy condition of the buccal mucosa, and a nervous disturbance which, in some cases, is apparently associated with a pruritus that causes the animals to bite or lacerate the skin. Diarrhea is frequently observed, limitation of food in the first week of the experiment being almost invariably necessary to control it. Diets containing only 18 per cent of dry egg white supplemented with yeast extract produce considerable growth in the early part of the experiment; some animals receiving such diets continue to grow slowly even after the appearance of marked symptoms of the disease but, eventually, growth ceases and the animals decline in weight and die.

The severity of the skin lesions is decreased and their character altered somewhat by the presence of a small amount of linseed oil or 18 per cent of butterfat or hydrogenated cottonseed oil in the diet. This is probably due to the correction of a deficiency of unsaturated fatty acids in the low fat diet rather than to any direct amelioration of the effect of the egg white. Even when the diets are supplemented with brewer's yeast which prevents the egg white disease, the skins of the rats receiving the low fat diets become dry and rough early in the experiment. This condition is mild, however, as compared to the condition produced by basic raw egg white diets which are low in fat. It is evident that with such diets the condition of the skin is affected by two distinct dietary factors.

Hypochlorhydria appears in the late stages of the disease but is seldom found before the other symptoms are well developed. Its late appearance does not indicate that it plays an important part in the causation of the disease. This is further indicated by the negative response of diseased animals to the administration of hydrochloric acid.

There is also a tendency for the hemoglobin concentration of the blood to decrease in the severe stages of the disease. This tendency is more apparent in the rats receiving the medium fat diets than in those receiving the low fat diets. It is probable that, in the latter, an anhydremia obscures any decrease in hemoglobin which may have occurred.

The abnormal symptoms are not produced by a diet which contains 18 per cent of egg white that has been coagulated by heat and extracted with 51 per cent alcohol. The symptoms are also prevented by the inclusion of such substances as liver, brewer's yeast, or the extracted residue of these substances in the raw egg white diet. They are not prevented by extracted casein, white corn, baker's yeast, extract of brewer's yeast, or the hydrolyzed residue of brewer's yeast. Liver extract is only partially preventive. Rats that have developed severe symptoms may be restored to an apparently normal condition in most cases by the feeding of brewer's yeast, extracted residue of brewer's yeast, dried liver, or milk; they cannot be restored by the feeding of extracted casein, gelatin, extract of brewer's yeast, or dilute hydrochloric acid.

It is possible that the discrepancy in the findings of Boas and of Parsons regarding the potency of yeast may be explained by differences in the yeasts used. Parsons apparently used a baker's yeast grown on a synthetic medium. In the experiments reported in this paper a baker's yeast had a very low potency, if it could be considered at all protective. On the other hand, a full grain-grown brewer's yeast was very effective, comparing favorably with beef liver.

The egg white syndrome presents features which are suggestive of a deficiency disease. However, the demonstration that the diet is innocuous if the egg white is coagulated and extracted (or, as Parsons has recently shown, if it is merely cooked) instead of fed raw, shows that, if a deficiency is present, it is due to poor digestion of the raw protein rather than to its composition. Bateman ('16) and others have shown that raw egg white is not well digested and that its presence

even inhibits the digestion of other proteins. The fact that the syndrome is produced by the addition of 18 per cent of raw egg white to a casein diet which alone would permit normal growth may possibly be due to the latter effect of the raw egg white. Nevertheless, it does not seem that the presence of raw egg white should affect the digestion of casein or gelatin more than that of brewer's yeast, liver, or the other proteins of milk. Moreover, animals tend to develop a tolerance to raw egg white which enables them to digest it more efficiently (Bateman, '16).

On the other hand, the typical syndrome develops only after the rats have received the raw egg white diets for several weeks, during which time considerable growth may have occurred. In this condition there certainly is no evidence of the development of a tolerance. The results may be explained on the basis of positive injury by the raw egg white more readily than on the basis of a deficiency. If this is the correct assumption, then it follows that the harmful factor in the raw egg white is antagonized by the substances which have been shown to be protective.

The symptoms of egg white injury are sufficiently similar to those produced by a deficiency of vitamin G to indicate the possibility that the harmful factor, if present, has an anti-vitamin G action. Since increasing the supply of vitamin G does not prevent the symptoms and since the vitamin G free residue of brewer's yeast or liver does prevent them, it is apparent that the theory of an anti-vitamin G action is untenable. Although increasing the supply of vitamin G in the egg white diets by the addition of extract B-G does not prevent the onset of symptoms, it increases the rate of growth. The vitamin G fraction of yeast extract apparently corrects a deficiency in the vitamin G fraction of the egg white. Further evidence of this supplemental effect is seen in group 39 where a marked stimulation of growth resulted from the use of extract B-G in addition to the extract from coagulated egg white.



The similarity of the symptoms in egg white disease, vitamin G deficiency, and pellagra is peculiarly interesting if one accepts the theory that native egg white contains a positive substance which is capable of producing such symptoms. It is evident that vitamin G deficiency is not the only condition in which pellagra-like symptoms may occur. The pigmentation and the dermatitis in egg white disease are even more typical of pellagra than are the symptoms of vitamin G deficiency. Numerous investigators have commented upon the variable character of the dermatitis in vitamin G deficiency. In this laboratory it has been observed that if a fuller's earth adsorbate obtained from extracts of brewer's yeast is used as a source of vitamin B in vitamin G deficient diets for rats, there is no extensive loss of hair, and generalized skin lesions are rarely seen; the only prominent symptoms are conjunctivitis, ulceration of the tongue, dermatitis in the nasal and oral regions, and edema, erythema, and exfoliation of epithelium of the feet. However, if the vitamin B adsorbate is prepared from white corn there is marked alopecia, and generalized skin lesions are frequent and severe. The experiments have not indicated whether the differences are due to other nutritive factors carried by the vitamin B preparation from brewer's yeast or to the presence of injurious substances in the preparation from corn.

#### SUMMARY

The use of raw egg white as the sole dietary source of protein for rats produced fur-like or woolly hair, alopecia, exfoliating dermatitis, hyperemia, skin hemorrhages, blepharitis, stomatitis, salivation, variable edema and erythema of the feet, symptoms of nervous disturbance, and finally hypochlorhydria and some anemia.

The results obtained from fresh egg white and dried egg white were similar when both were fed in the raw form.

The use of extremely high concentrations of protein was not essential to the production of the disease; diets containing only 18 per cent of raw egg white (dry basis) caused severe symptoms.

The skin lesions were more severe on low fat diets than on diets containing 18 per cent of butterfat or hydrogenated cottonseed oil or 0.20 ml. of linseed oil per rat daily.

If the diet contained not more than 18 per cent of raw egg white (dry basis) the symptoms were prevented by the inclusion of brewer's yeast, dried liver, or the extracted residue of brewer's yeast or liver in the diet but not by the inclusion of extracted casein, extract or hydrolyzed residue of brewer's yeast, white corn, or baker's yeast; liver extract was more effective than yeast extract but was only partially preventive.

Well developed cases of the disease were apparently cured by brewer's yeast, dried liver, milk, or extracted residue of brewer's yeast but were not improved by extracted casein, gelatin, extract of brewer's yeast, or dilute hydrochloric acid.

Coagulation of fresh egg white by heat and extraction of the coagulum with 51 per cent alcohol rendered it innocuous.

The concentrated extract of egg white had a slight harmful effect but failed to produce the severe symptoms caused by raw egg white. This extract, as a source of vitamin G, was less effective than an extract of brewer's yeast.

The data indicate a positive harmful factor in raw egg white which is antagonized by the protective substances rather than the existence of a deficiency.

The results are apparently not due to an anti-vitamin G action of the harmful factor, although many of the symptoms are similar to those produced on certain vitamin G deficient diets and even more similar to those of pellagra.

## LITERATURE CITED

- BATEMAN, W. G. 1916 The digestibility and utilization of egg proteins. *J. Biol. Chem.*, vol. 26, p. 263.
- BOAS, M. A. 1927 The effect of desiccation upon the nutritive properties of egg white. *Biochem. J.*, vol. 21, p. 712.
- BURR, G. O., AND M. M. BURR 1929 A new deficiency disease produced by the rigid exclusion of fat from the diet. *J. Biol. Chem.*, vol. 82, p. 345.
- 1930 On the nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.*, vol. 86, p. 587.
- BURR, G. O., M. M. BURR AND E. S. MILLER 1932 On the fatty acids essential in nutrition. *J. Biol. Chem.*, vol. 97, p. 1.
- CHICK, H., AND A. M. COPPING 1930 The composite nature of the water-soluble vitamin B. III. Dietary factors in addition to the anti-neuritic vitamin B<sub>1</sub> and the anti-dermatitis vitamin B<sub>2</sub>. *Biochem. J.*, vol. 24, p. 1764.
- CHICK, H., A. M. COPPING AND M. H. ROSCOE 1930 Egg white as a source of the anti-dermatitis vitamin B<sub>2</sub>. *Biochem. J.*, vol. 24, p. 1748.
- CHICK, H., AND M. H. ROSCOE 1929 A method for the assay of the anti-neuritic vitamin B<sub>1</sub> in which the growth of young rats is used as a criterion. *Biochem. J.*, vol. 23, p. 498.
- FINDLAY, C. M., AND R. O. STERN 1929 Syndrome in rat resembling pink disease in man. *Arch. Dis. Childhood*, vol. 4, p. 1.
- FIXSEN, M. A. BOAS 1931 The effect of desiccation upon the nutritive properties of egg white. II. *Biochem. J.*, vol. 25, p. 596.
- MCAMIS, A. J., W. E. ANDERSON AND L. B. MENDEL 1929 Growth of rats on 'fat-free' diets. *J. Biol. Chem.*, vol. 82, p. 247.
- PARSONS, H. T. 1931 The physiological effects of diets rich in egg white. *J. Biol. Chem.*, vol. 90, p. 351.
- PARSONS, H. T., AND E. KELLY 1933 The effect of heating egg white on certain characteristic pellagra-like manifestations produced in rats by its dietary use. *Am. J. Physiol.*, vol. 104, p. 150.
- 1933 The character of the dermatitis-producing factor in dietary egg white as shown by certain chemical treatments. *J. Biol. Chem.*, vol. 100, p. 645.
- PARSONS, H. T., J. G. LEASE AND E. KELLY 1933 The cure of dermatitis due to egg white by various foodstuffs. *J. Biol. Chem.*, vol. 100, p. lxxvii.
- RINGROSE, A. T., L. C. NORRIS AND G. F. HEUSER 1931 The occurrence of a pellagra-like syndrome in chicks. *Poultry Sci.*, vol. 10, p. 166.
- SALMON, W. D. 1931 Rats on casein diet develop a condition resembling the egg white syndrome of Boas. 42nd An. Rpt., Ala. Exp. Sta., p. 33.
- 1931 An improved method for extraction of vitamin B from a fuller's earth adsorbate. 42nd An. Rpt. Ala. Exp. Sta., p. 43.

# STORAGE OF VITAMIN A IN CATTLE <sup>1</sup>

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## ONE FIGURE

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In previous publications (Hart et al., '32, '33a, '33b) we have shown that cattle on the range in California are subject to low vitamin A intake during the dry season. The length of the drought period varies from year to year, with a corresponding variation in the manifestations of vitamin A deficiency. Under natural conditions a single deficiency uncomplicated by other factors rarely occurs. This fact, coupled with the variation in the syndrome of vitamin A deficiency, rendered observations difficult to interpret without fuller knowledge of mild evidences and of the complications that arise as the symptoms become more acute, finally ending in death of the animals.

The three most important manifestations of vitamin A deficiency in cattle are expulsion of the fetus prematurely or dead at term, severe diarrhea in newborn calves, and ophthalmia in young growing animals. These conditions are easily confused with bovine infectious abortion, white scours in calves, and infectious keratitis. The differential diagnosis of these conditions has been described by us (Hart and Guilbert, '33 b).

<sup>1</sup> This report is part of an investigation on the relation of nutrition to reproduction in livestock, which became cooperative with the United States Bureau of Animal Industry, July 1, 1929.

The ability of cattle to store vitamin A in times of abundance against periods of privation is extremely important under range conditions in California. It is valuable therefore to have some knowledge of the extent of this reserve in order that supplements may be most economically supplied to prevent deficiency.

Halverson and Sherwood ('30) have definitely demonstrated that the so-called cottonseed poisoning in cattle is primarily caused by the vitamin A deficiency of rations consisting of cottonseed hulls and meal or combinations of these feeds with white corn, oats or beet pulp.

Experience in commercial feed lots shows that heavy feeding of steers on cottonseed meal and hulls for periods in excess of 100 days is liable to cause blindness, loss of appetite, and rapid loss of weight in many animals. A difference in the time of encountering these symptoms, depending on whether or not the steers are brought into the feed lot from good feed (which usually means green feed) or poor feed, is also recognized. In one experiment Halverson and Sherwood ('30) report the appearance of various eye lesions and of blindness without visible lesions, in a large percentage of steers 88 days after being fed in dry lot on an exclusive ration of cotton-seed hulls and meal. These authors do not mention the previous feeding of these animals. According to other experiments which they report, dairy heifers averaging less than a year of age may subsist on such a ration for 200 days before the onset of acute symptoms. In nearly all cases, however, death ensued soon thereafter unless the ration was changed.

Mead and Regan ('31) reported the development of avitaminosis A in calves 1 to 3 months after being changed from whole milk and grain to a concentrate mixture low in vitamin A. No roughage was fed.

These and other data indicate variability in the onset of deficiency symptoms, depending on stage of maturity, lactation, previous feed supply and degree of deficiency of the rations.

This paper primarily considers the time required to deplete non-lactating cattle that have had ample opportunity to store vitamin A. The animals used were beef steers and heifers continuously supplied with green feed until placed on experiment. The storage of vitamin A in livers was studied by means of the blue color reaction with antimony trichloride.

#### EXPERIMENTAL

*Technic used in color test for vitamin A.* The use of the Carr-Price ('26) reaction for quantitative estimation of vitamin A offers increased opportunity to extend knowledge on vitamin A problems in the field of livestock production, even though it may have limitations as a precise method of vitamin A assay. The examination of the vitamin A content of liver tissue by this method is an excellent diagnostic aid in cases of suspected natural occurrence of the deficiency, especially in borderline cases where definite clinical symptoms are lacking.

It is recognized that a number of substances may react with antimony trichloride to form a blue color. In a rather wide experience, however, we have obtained at most a trace of blue color; more commonly none from the liver tissue of animals known to be depleted of vitamin A. On the other hand, we have never failed to obtain strong reactions with extracts of liver tissue from animals which were abundantly supplied.

In sampling liver tissue of large animals, two slices are generally taken from different parts of the organ. We have found no difference in the various cross sections of the liver. Thin peripheral slices, however, have proved less potent in vitamin A than sections nearer the center. The samples are ground in a food chopper and mixed; then duplicate 10 to 20 gm. samples are weighed into 250 cc. Erlenmeyer flasks.

The digestion and extraction of liver tissue closely follows the method of Moore ('30). Twenty to forty cubic centimeters of 5 per cent KOH are poured into the flask containing the liver tissue, which is then brought into solution by heating for a few minutes in a boiling water bath. The strength

of KOH is of minor importance; we obtain the same results with 5, 20 and 50 per cent KOH. The stronger alkali solutions are used when complete saponification is desired.

After the liver tissue has been brought into solution and cooled, the contents of the flask are transferred to a separatory funnel, and 20 to 30 cc. of 50 per cent ethyl alcohol added. The solution is extracted twice with ethyl ether, using a total of three to four volumes compared with the solution to be extracted. After the alkaline layer has been drawn off, the ether solution is washed free from alkali with distilled water, and is transferred to a flask containing 5 to 10 gm. of anhydrous sodium sulfate. After a vigorous shaking it is allowed to stand for a few minutes. The solution is then decanted, and the sodium sulfate washed twice with small volumes of fresh ether. Next the ether is distilled off, care being taken thereafter not to subject the residual material to heat. The flask containing the liver oil (or only the unsaponifiable fraction) is then placed in a vacuum desiccator. If the material is to stand for some time under vacuum, the air is first washed out with  $\text{CO}_2$ . A few minutes under vacuum, however, suffice to evaporate remaining traces of ether, the water having been largely removed by the previous contact with anhydrous sodium sulfate. The residue is dissolved in chloroform to a suitable volume. If the liver oil in question is suspected of being low in vitamin A, we use as small a volume as possible (1 to 2 cc.). For normal liver tissue a dilution with chloroform to 10 cc. is satisfactory. Since the intensity of the blue color formed with antimony trichloride depends both upon the concentration of the vitamin or precursors and upon the concentration of the reagent, the test solution must be kept to small volume. The test should be conducted as soon as possible after the material is dissolved in chloroform for the vitamin appears to be unstable in chloroform solution. We have found potent samples to have changed in standing overnight to such an extent that only the red or reddish-brown color developed.

Quantitative estimations are made by diluting the test solution until 0.05 to 0.2 cc. with 2 cc. of antimony trichloride reagent produces a color that approaches the vanishing point. The quantity of the solution required may then be calculated back to the amount of liver tissue it represents.

In our work a unit is defined as the amount of chromogen in 0.05 to 0.2 cc. of chloroform solution which will give the faintest detectable blue color with 2 cc. of antimony trichloride reagent. We have found that 1 to 1.5 gamma of crystalline carotene (S.M.A. Corporation. M.P. 166° to 168°) is equivalent to 1 color unit. If 0.5 gamma of carotene is considered 1 rat unit, then 1 color unit is approximately 2.5 R.U. Since vitamin A produces ten to twenty times as much color with antimony trichloride as carotene (Moore, '33) and since he has further shown that vitamin A and carotene are equal biologically, 1 color unit in terms of vitamin A would be equivalent to 0.1 to 0.25 R.U. Our unit should not therefore be confused with blue units based upon the Lovibond tintometer.

Reasonably good agreement between duplicate samples among three independent workers in this laboratory has been obtained by this method. Since adopting this technic, we have noted a paper by Andersen and Nightingale ('29), who employed a similar dilution procedure for quantitative tests for vitamin A in butter, margarine and other fatty foods.

In order that judgment of the end point may not vary too much because of light variations, comparison is made against a solution of  $\text{CuSO}_4$  equivalent to 0.5 mg. of copper per cubic centimeter, kept in a sealed tube of the same diameter as that used for the test solutions. The antimony trichloride reagent used is a saturated solution in C.P. chloroform at room temperature, the crystals having first been washed with a little chloroform. About 30 gm. of antimony trichloride to 100 cc. of chloroform are required.

*Animals and rations used.* Twelve beef steers, varying in age from 9 to 20 months, were selected in September, 1932, from the University beef herd. All these animals had grazed



on native pasture during the previous spring. When the grass dried they were either pastured on irrigated alfalfa or sudan grass or fed in dry lot on a ration consisting of concentrates (barley, oats and wheat bran) fresh green corn, alfalfa and sudan grass hay until placed in the experiment. The conditions may therefore be considered ideal for storage of vitamin A. Four animals constituting group 1 were killed at the beginning, and samples of the livers were obtained. Six head, constituting group 2, were placed on a low vitamin A ration consisting of dried molasses beet pulp 70 per cent, rolled barley 14 per cent, cottonseed meal 15 per cent, and  $\text{CaCO}_3$  1 per cent. The two remaining animals, constituting group 3, were fed the same grain ration as those in group 2, but each received in addition 1 pound daily of high-quality field-cured alfalfa hay. The average amount of feed consumed was 15 pounds of the concentrate mixture daily. No apparent difficulty was encountered from lack of roughage in the ration. The steers in groups 2 and 3 were killed after varying intervals on these rations and the livers were examined for their content of vitamin A. The first pair from group 2, nos. 427 and 441, were killed after 63 days; the second pair, nos. 424 and 445, after 121 days, and the third pair, nos. 444 and 428, after 282 days, in an advanced stage of vitamin A deficiency. Steers 422 and 425 of group 3 were autopsied after 127 days.

The data obtained from the liver samples of these animals are presented in table 1.

Table 1 shows the rather uniform storage in the livers of the group 1 animals and the progressive depletion of the reserves in the animals of group 2 as the time on the deficient diet advanced. The feeding of 1 pound of high quality alfalfa hay to group 3 apparently had little effect upon the maintenance of reserve in their livers. We have some evidence, however, that this quantity suffices to delay the appearance of clinical symptoms and may be valuable under certain conditions in supplementing vitamin A deficient range.

The onset and progress of clinical symptoms of vitamin A deficiency in steers 428 and 444 of group 2 were as follows:

On June 7th, 225 days after being placed on the experiment, no. 444 showed excessive lacrimation, especially in bright sunlight, and on June 30th showed night blindness for the first

TABLE 1

*Depletion in storage of vitamin A in the livers of steers fed a ration deficient in this essential*

GROUP NO.	STEER NO.	AGE WHEN SLAUGHTERED	BREED	INITIAL WEIGHT	FINAL WEIGHT	FEEDING PRIOR TO SLAUGHTER	UNITS PER GRAM OF LIVER <sup>1</sup>
		<i>months</i>					
I	419	17	Crossbred	....	1050	Grain, green fodder and hay	500
	421	17	Hereford	....	925	Green pasture	500
	437	12	Hereford	....	820	Green pasture	500
	417	19	Shorthorn	....	800	Grain, green fodder and hay	830
II	427	20	Shorthorn	1012	1100	63 days on ration deficient in vitamin A	250
	441	13	Aberdeen Angus	765	845	121 days on ration deficient in vitamin A	420
	424	21	Shorthorn	1020	1165	121 days on ration deficient in vitamin A	160
	445	13	Shorthorn	600	800	282 days on ration deficient in vitamin A	50
	444	19	Shorthorn	630	1050	282 days on ration deficient in vitamin A	Trace?
	428	25	Shorthorn	1020	1280	127 days on ration deficient in vitamin A + 1 pound alfalfa daily	Trace?
III	422	20	Crossbred	1080	1195	127 days on ration deficient in vitamin A + 1 pound alfalfa daily	80
	425	20	Crossbred	955	1090	127 days on ration deficient in vitamin A + 1 pound alfalfa daily	50

<sup>1</sup> See definition of units in section on technic.

time. He manifested temporary blindness when driven around the corral in semi-darkness. On June 23rd, no. 428 had a slight nasal discharge, and his nose was dry and scaly. On July 4th, he exhibited night blindness and muscular incoordination. On July 7th, when the animals were tested in semi-darkness, both steers ran into the fence and posts in the

corral repeatedly, although normal animals had no difficulty in avoiding them. Finally, the steers came together head on; and, although the force of the impact was not great, no. 428 dropped as though felled by a stunning sledge, rolled to his back, and quivered with his legs extended. After a few seconds he was able to regain his feet.

From this time on, sight became more and more defective until there was considerable impairment even in daylight. The degree of impairment varied from one time to another, as did also the muscular incoordination, which took the form of staggering from side to side when these steers were driven about the lot. At times the rear legs appeared most affected. On July 12th, both steers were discharging mucus from their nostrils continuously. Tears flowed from their eyes, keeping the hair wet down the sides of their faces. Aside from slight inflammation and edema of the nictitans membranes of steer 444, no visible eye lesions were noted. The corneas did not become clouded in these cases.

On July 15th, the nose of steer 444 appeared slightly twisted laterally, suggesting partial facial paralysis. On July 26th, steer 428 showed definite facial paralysis: the left ear drooped and flopped when he moved about, and the left eye appeared somewhat sunken. During this time appetite remained good, the steers regularly consuming their daily allowance of 15 pounds of concentrate mixture. On July 29th, however, a mild diarrhea of intermittent nature appeared, and part of the feed was not eaten. Up to this point the general appearance of the animals had been good, but in the next few days the decline was rapid. The nasal discharge, sight impairment, and muscular incoordination became more marked. The hair began to appear rough, appetite failed, and weight rapidly declined. On August 4th the steers were slaughtered after 282 days on the deficient ration.

On post mortem examination the internal organs appeared normal. Both steers had a dressing percentage of about 65, indicating a high degree of fatness. The fat was pure white, whereas the fat of those killed at 60 to 120 days was a creamy

yellow, and that of steers killed at the beginning of the experiment carried considerable pigment. This experiment shows that carotene, the principal pigment of beef fat, may be withdrawn from the adipose tissue during vitamin A privation without a coincident reduction of the fat reserve. The carotene in the fat of cattle undoubtedly constitutes a significant part of the total vitamin A reserve. All these animals were fat enough for slaughter when placed on the experiment and consequently may have had more reserve than thin animals on the same kind of feed.

With antimony trichloride reagent the non-saponifiable matter from 20-gm. samples of the livers of these steers gave a faint violet color, probably caused by a trace of blue color combined with the red commonly encountered when the extract is devoid of vitamin A.

The weight curves of these steers are presented in figure 1. These show nearly uniform weight increases at the rates of 1 and 1.3 pounds daily, respectively, up to the time when vitamin A reserves were practically exhausted and the appetites of the animals had failed.

*Storage of vitamin A in heifers.* This was an experiment to study the effects of nutritional deficiencies during gestation. The basal ration used consisted of dried molasses beet pulp, wheat straw, and calcium carbonate; it was definitely deficient in protein, phosphorus, and vitamin A. Heifers 15 to 20 months of age, free from infectious abortion, were placed on experiment soon after becoming pregnant. Two of them, nos. 426 and G 13, were fed the basal ration. The former which came from the University beef herd had abundant vitamin A in her ration until placed on experiment. After being on this ration for about 8 months, she sustained marked loss in weight, declined to low levels in blood phosphorus, and manifested marked osteophagia and other forms of pica. She produced at term a live but undersized calf and developed no clinical evidence of vitamin A deficiency. Because her milk supply was meager, growth of the calf was extremely limited.

The calf lived for 5 months upon this meager milk supply supplemented by straw and concentrate mixture eaten with the mother. Its weight increased only 25 pounds throughout the period. While general under-nutrition contributed to the death of this calf, definite symptoms of vitamin A deficiency were recorded at post mortem. The crystalline lenses of both eyes were clouded, although the corneas were not affected.

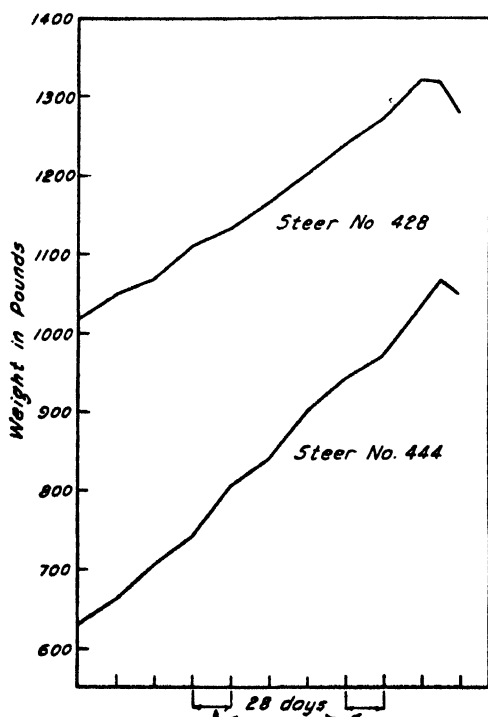


Fig.1 Weight curves of steers 428 and 444, showing continuous gains until the time when vitamin A reserves were practically exhausted and the appetites of the animals had failed.

The right lung had pneumonic areas in which were nodules containing air and pus cells similar to other cases examined involving vitamin A deficiency (Hart and Guilbert, '33 b). The extract from 20 gm. of the liver tissue, concentrated to small volume, gave no test for vitamin A. Mild diarrhea had been noted at intervals prior to death. It is interesting that

the dam of this calf has continued for 14 months without showing symptoms of vitamin A deficiency. We venture the explanation that the multiple deficiencies of the diet resulting in the animal bordering on starvation greatly retarded the depletion of reserves as compared to the steers of comparable age on a more complete ration.

The second heifer, no. G 13, was range raised and was purchased in the fall after having been on dry forage during the summer. She was placed on pasture containing some green feed and also received hay for 6 weeks before going on the basal ration. This heifer exhibited marked night blindness after 195 days. She aborted the two hundred and twenty-ninth day of pregnancy, after being on the ration 205 days. Upon slaughtering, her fat was found to be white, and extensive abscess formation was present in one lung. A sample of her liver gave a color test of only 2 units per gram. The liver tissue of the fetus gave no color test for vitamin A.

Heifer 429, from the University herd was fed 1 pound of high-quality field-cured alfalfa hay in addition to the basal ration. She carried her calf to term (283 days) but parturition was difficult. The cow was found exhausted in labor in the morning with the fetus anteriorly presented in the pelvic cavity. Strong traction was necessary for its delivery. It was a large calf weighing 83 pounds and probably died during parturition. Its liver extract showed a trace of blue color with antimony trichloride.

Four other heifers calved normally at term. The diets of two of them, nos. G54 and 440, were modified to eliminate phosphorus deficiency and in the other two, nos. 410 and 440, to eliminate phosphorus and protein deficiency; but in all cases practically the sole source of vitamin A was 1 pound of alfalfa hay daily.

The calves from nos. G54 and 410 were normal until 7 or 8 days of age when they developed a diarrhea, which became so severe that white-colored liquid feces were discharged at frequent intervals. Suspecting that inadequacy of vitamin A might be involved, cod liver oil of high biological value was

given by mouth in 10 cc. doses daily to the calf from no. G54 which was most severely affected. The other calf was kept as a control. Improvement in the fecal discharges and physical appearance of the calf receiving cod liver oil was evident by the fourth day, whereas the control calf was rapidly becoming worse. Cod liver oil was then administered in the same dose and improvement followed, both calves returning to normal in 8 or 9 days when the oil was discontinued. The calves grew at nearly normal rate for 2 months, at the end of which time the calf from no. G54 showed marked night blindness and again developed severe diarrhea. A day or two later the calf from no. 410 also had diarrhea. The latter was again kept as a control and the former treated with cod liver oil. The night blindness and diarrhea disappeared after 4 days. The control calf continued to have diarrhea for several days, then recovered without treatment. For 10 days prior to the second onset of diarrhea these calves were given 1 pound each of dried molasses beet pulp. Since they were fed together it is possible that the G54 calf may have eaten less than her share after the onset of diarrhea and overeating may have contributed to the diarrhea in the calf kept as a control.

Heifer no. 438 had an apparently normal calf which weighed 73 pounds at birth. This calf developed severe diarrhea the third day following parturition and was given 10 cc. daily of cod liver oil. It declined rapidly, however, developed pneumonia and died 3 days later. Post mortem examination showed mild general enteritis and broncho-pneumonic areas in the left lung. The antimony trichloride test for vitamin A in the liver gave a relatively high value of 125 units per gram, presumably because of the cod liver oil feeding.

Heifer no. 440 calved normally. The calf weighed 82 pounds but appeared weak. After being assisted to its feet the calf nursed, but on the second day developed severe diarrhea. The appetite declined and it nursed very little. No treatment was given. After 5 days improvement was noted, appetite returned and within the next week it grew active and strong.

The mother was milked out regularly during the period when the calf did not take all the milk, as was also done with heifer no. 438.

Milk samples were taken from these heifers at varying times and the antimony trichloride test was applied to the unsaponifiable fraction of the milk fat. The results are presented in table 2. The first samples from nos. G54 and 410 were taken at the time their calves developed diarrhea. The apparently negative result obtained for no. 410 must be con-

TABLE 2  
*Antimony trichloride color test on milk fats*

DESCRIPTION OF SAMPLE	UNITS PER GRAM OF MILK FAT				
	No. G54	No. 410	No. 438	No. 440	Dairy herd
Colostrum, 1st day				360	
Colostrum, 2nd day				155	
Colostrum, 3rd day			150	120	
Milk, 7th day			20		
Milk, 10th day	Negative	?	10		
Milk, 14th day			20	7	
Milk, 24th day			15		
Milk, 43rd day				14	
Milk, 3½ months	Trace	7			
Milk, 4 months	Negative	Trace			
Milk, 5 months	Negative	Negative			
Composite sample—winter milk					27
Composite sample—winter milk					33
Composite sample—winter milk					35

sidered questionable since the butter fat content of the milk was very low and some difficulty was encountered with this sample. All other results have been run under conditions known to give consistent results on normal milk. The fact that these animals were not accustomed to being milked and had to be restrained discouraged us from taking samples frequently in the early part of the experiment.

Heifer G54 was range raised and had the same previous history as heifer G13 which developed clinical symptoms of vitamin A deficiency and aborted. The fact that the former animal received 1 pound of alfalfa hay daily containing ap-



proximately 16 mg. of carotene possibly explains the normal completion of her gestation. This amount did not, however, provide sufficient for her to secrete detectable quantities in the milk, nor to prevent the onset of symptoms of vitamin A deficiency (night blindness) in the calf at about  $2\frac{1}{2}$  months of age, even after it had been given 10 cc. daily of cod liver oil from the eleventh to the twentieth day after birth.

The other three heifers were from the University herd and had ample opportunity to store the maximum vitamin A for their age, prior to the experimental period. Table 2 shows that after the colostrum period, their milk fat contained less than half the vitamin A found in winter milk samples from the dairy herd. The butter fat of the experimental heifers was practically colorless, while the dairy herd samples contained considerable pigment. Since carotene gives much less blue color with antimony trichloride in relation to its biological value than does vitamin A, the difference between the samples shown in table 2 is minimized.

None of these heifers, except G13, have shown clinical evidence of vitamin A deficiency up to 6 months after parturition.

The relation between occurrence of diarrhea in the calves to the inadequacy of the diet of the dams is not as yet clear. The regularity of its occurrence appears significant as well as the recurrence of the disorder simultaneously with night blindness and the apparent response to vitamin A therapy in the case of the calf from no. G54. Since, as will be shown in a later section of this paper, the reserves at birth are low even in calves from cows having abundant storage, and since the liver of one calf that died during parturition in this experiment had only a trace of vitamin A, it is possible that the calves in utero received an inadequate supply, even though the colostrum of at least two of the dams was found to be comparatively rich in the vitamin. The relating of diarrhea to vitamin A deficiency by other investigators and our own observations in the naturally occurring cases on the range support this view.

*Storage of vitamin A in the livers of mature cows.* Liver samples were obtained from two aged cows from the University beef cattle herd. These animals were 11 and 12 years old, respectively, and throughout life had access to green pasture for at least 9 months of every year. During the remainder of the year they received a ration of alfalfa hay and silage. Both cows had produced calves regularly. No. 63 was practically dry at the time of slaughter, having nursed a calf for a period of about 8 months. No. 75 had not been producing milk for several months prior to slaughter. Both cows were excessively fat.

Ten additional samples of liver were obtained at a local slaughter house from a lot of cows that had been for 7 months on range feeds deficient in vitamin A. During the early part of the dry feed period the cattle grazed native pasture. Later they were pastured on barley stubble and during the last 6 weeks before slaughter were fed in addition to dry pasturage, 10 pounds of barley and a small allowance of sesame meal daily. These cows were all well along in years and were not lactating. Most of them probably had not produced calves the previous spring. The fat on the carcasses of these animals contained considerable yellow pigment.

The results of the tests on the livers are presented in table 3. Cows nos. 63 and 75, from the University herd, had a concentration of vitamin A in their liver tissues approximating ten times that found in the growing steers 12 to 20 months of age. The liver tissues of these cows were comparable in concentration of vitamin A to values found for high potency cod liver oil. The non-saponifiable fractions of these livers are therefore an exceedingly concentrated source of vitamin A. The non-saponifiable matter was fractionated by the phase method. As shown in table 3, nearly all of the chromogenic value was found in the 85 per cent methyl alcohol fraction. Practically all of the yellow pigment, however, remained in the petroleum ether fraction, showing it to be carotene. Since the methyl alcohol fraction was nearly colorless, vitamin A must have been responsible for practically all of the blue color formed by the reaction with antimony trichloride.

The liver storage in the range cows that were killed after 7 months on dry feed varied widely. The highest storage was only one-fifth that found in the University cows. The four samples that were lowest in vitamin A (80 to 200 units per gram) indicate storage sufficient to protect the animals for several months if the experience with the steers previously cited may be taken as a criterion. Since these cows were mature and had not been lactating for some time, it is reasonable to expect slow depletion of reserve. The varia-

TABLE 3  
*Storage of vitamin A in the livers of mature cows*

DESIGNATION OF SAMPLE	UNITS PER GRAM OF LIVER		
	Total (direct determination)	Petrol ether fraction	85 per cent methyl alcohol fraction
No. 63 University herd	4500	65	4400
No. 75 University herd	5000	80	5000
No. 1 Range cow	800		
No. 2 Range cow	500		
No. 3 Range cow	550		
No. 4 Range cow	500		
No. 5 Range cow	80		
No. 6 Range cow	200		
No. 7 Range cow	125		
No. 8 Range cow	400		
No. 9 Range cow	80		
No. 10 Range cow	1000		

tions found may be related to past history of the animals of which we have no record.

*Storage of vitamin A in the livers of calves.* Busson and Simmonet ('32) have reported the livers of newborn pups to be low in vitamin A even when the maternal liver contained large amount. Dann ('32) has also presented data showing the livers of rats and rabbits to be relatively low at birth, the storage being increased during the nursing period when the mothers were fed diets rich in carotenoids.

The livers of several calves have been tested for their content of vitamin A. The mothers, except the one noted in

table 4, had been on rations containing abundant vitamin A (good quality alfalfa hay, silage and pasture). The results are presented in table 4.

Since we have obtained values as high as 5000 units per gram from liver samples of older animals, it is evident from the data in table 4 that the storage in the liver of newborn calves is relatively low regardless of the storage in the dam. The liver extract of calves is practically colorless, and presumably the chromogenic substance is preformed vitamin A.

TABLE 4  
*Vitamin A content of the livers of calves*

DESCRIPTION OF CALF	UNITS PER GRAM OF LIVER
Shorthorn born dead. Mechanical difficulties at parturition	42
Hereford, twin calves. Died at birth 10 days premature	12
Jersey. Killed 18 hours after birth. Had mother's milk	14
Jersey. Killed 36 hours after birth. Had mother's milk	10
Jersey. Killed 3 days after birth. Had mother's milk	5
Liver from cow receiving 75 cc. cod liver oil daily as sole source of vitamin A.	250
Liver of 7½ months' fetus from above cow	100
Holstein calf. Born dead	25

In normally-fed bovines, on the other hand, the liver extracts carry considerable carotene. These results agree with those of Palmer ('22), who found that tissues of newborn calves were practically free from carotenoids.

#### DISCUSSION

The data presented in this paper show that there is a variation in the time in which vitamin A deficiency may be manifested in animals on a deficient diet. The principal factors are storage reserves and the production requirements of the animals. Lactation is probably a greater strain on reserve supply than is gestation. During the period of active growth there is also a relatively greater requirement and less storage than when adult size is attained. The low reserves in the liver of newborn calves and other animals is evidence that the

pregnant mother is not able to mobilize this essential in the tissues of the fetus much faster than it is utilized. Evidently, therefore, the fetus will be the first to suffer when the borderline of deficiency is reached. Drummond, Coward and Watson ('21) showed that colostrum has a higher biological value and is richer in carotenoids than later milk from the same animal—a fact also shown by our chemical determinations. This is a very interesting parallelism to the concentration of euglobulin, carrying immune bodies in this important physiological secretion for the newborn, while, on the other hand, iron, which can readily pass to the fetus in utero, is stored in excess against a low intake during the milk-drinking period. Mead's (unpublished data) studies on the raising of dairy calves at this station have brought to our attention the importance of colostrum from the vitamin A standpoint.

Animals on the range that are able to obtain only a sub-maintenance intake of feed, utilize vitamin A less rapidly than animals on a supermaintenance and otherwise complete diet. Hence night blindness, ophthalmia and other acute evidences of deficiency are more liable to be found where range feed is being supplemented with vitamin A deficient concentrates than on range feed alone.

The storage of vitamin A during the green feed season is sufficient to carry range animals safely through dry-feed periods of ordinary duration. When, however, early drying of forage in the spring is coupled with late rains in the fall, and especially when two such seasons occur in succession, reserves may be completely depleted and clinical symptoms appear.

In supplementing the range, one must consider the vitamin A reserve of the animals and the length of the deficiency period in order to meet all the deficiencies. Cottonseed cake, ideal as a protein and phosphorus supplement, must itself be supplemented with alfalfa or other sources of vitamin A when this deficiency appears. Early recognition of mild evidence of vitamin A deficiency makes it possible to avoid serious loss.

## SUMMARY

Clinical symptoms of vitamin A deficiency were evident in two beef steers after 225 to 240 days on a ration of dried molasses beet pulp, rolled barley, cottonseed meal and calcium carbonate. Reserves were practically exhausted, and the animals were in critical condition after 282 days, at which time they were autopsied. The gradual depletion of reserves with advancing time was followed by estimating the vitamin A potency of the liver oil of steers autopsied after varying periods on the deficient ration, the Carr-Price color reaction being used. According to evidence presented, the carotene in the adipose tissue, which constitutes a part of the vitamin A reserve, may be withdrawn during vitamin A privation without coincident withdrawal of the depot fat. All the steers used had ample opportunity to store vitamin A before the experimental period.

The expulsion of the fetus before it is viable, in the absence of infectious abortion, is described. Calves from heifers having a restricted intake of vitamin A during gestation developed a severe diarrhea at 2 to 8 days of age. The milk of the dams was shown to be deficient or subnormal in vitamin A. One calf exhibited marked night blindness although no clinical evidence of vitamin A deficiency has appeared in the dams up to 6 months following parturition. The liver tissue of mature beef cows, reared under favorable conditions, was found to have a concentration of vitamin A approximating that of high potency cod liver oil. The storage in the livers of newborn calves from cows receiving abundant vitamin A was found to be relatively low. Attention is called to the low concentration of vitamin A in the livers of calves at birth and to its high concentration in colostrum milk. This parallels the low concentration of globulins in the serum of newborn calves and their high concentration in the colostrum.

In these experiments the manifestations of vitamin A deficiency occurring under natural conditions on the range, which were reported in previous publications (Hart et al., '33 a, '33 b) have been produced under controlled conditions. The relation of these findings to problems in range cattle in California is discussed.

## LITERATURE CITED

- ANDERSON, A., AND E. NIGHTINGALE 1929 Test for vitamin A in margarine, butter and other fatty foods. *J. Soc. Chem. Ind.*, vol. 48, p. 139.
- BUSSON, A., AND H. SIMMONET 1932 Variation de la réserve en vitamine A du foie suivant l'âge chez le chien. *C. R. Soc. Biol.*, T. 109, p. 1253.
- CARR, F. H., AND E. A. PRICE 1926 Color reactions attributed to vitamin A. *Biochem. J.*, vol. 20, p. 497.
- DANN, W. J. 1932 The transmission of vitamin A from parents to young in mammals. *Biochem. J.*, vol. 26, p. 1072.
- DEUMMOND, J. C., K. H. COWARD AND A. F. WATSON 1921 Researches on vitamin A. VII. Notes on the factors influencing the value of milk and butter as sources of vitamin A. *Biochem. J.*, vol. 15, p. 540.
- HALVERSON, J. O., AND F. W. SHERWOOD 1930 Investigations in the feeding of cottonseed meal to cattle. *North Carolina Agr. Exp. Sta. Tech. Bul.* 39, pp. 1-158.
- HART, G. H., H. R. GUILBERT AND H. GOSS 1932 Seasonal changes in the chemical composition of range forage and their relation to the nutrition of animals. *Calif. Agr. Exp. Sta. Bul.* 543, pp. 1-60.
- HART, G. H., S. W. MEAD AND H. R. GUILBERT 1933 a Vitamin A deficiencies in cattle under natural conditions. *Proc. Soc. Exp. Biol. and Med.*, vol. 30, p. 1230.
- HART, G. H., AND H. R. GUILBERT 1933 b Vitamin A deficiency as related to reproduction in range cattle. *Calif. Agr. Exp. Sta. Bul.* 560, pp. 1-30.
- MEAD, S. W., AND W. R. REGAN 1931 Deficiencies in rations devoid of roughage for calves. *J. Dairy Sci.*, vol. 14, p. 283.
- MEAD, S. W. *Calif. Exp. Sta.*, unpublished data.
- MOORE, T. 1930 Vitamin A and carotene V. The absence of liver oil vitamin A from carotene. VI. The conversion of carotene to vitamin A in vivo. *Biochem. J.*, vol. 24, p. 692.
- 1933 The relative minimum doses of vitamin A and carotene. *Biochem. J.*, vol. 27, p. 898.
- PALMER, L. S. 1922 Carotinoids and related pigments. *The Chem. Cat. Co.*, p. 268. New York.

# VITAMIN A STORAGE IN THE LIVERS OF TURKEYS AND CHICKENS

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## ONE FIGURE

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Under conditions commonly prevailing in commercial poultry production in California, supplying vitamin A is an important problem. Since fresh green feed is frequently limited, definite quantities of alfalfa meal or leaf meal are fed primarily to furnish this dietary essential. Nevertheless, symptoms of vitamin A deficiency sometimes occur in birds on rations that are generally considered adequate. Avitaminosis-A occurs also in range raised turkeys during the drought period when the food consists largely of seeds, dried herbage and insects. Under these conditions exact feeding history is often not available, and the manifestations are varied and indefinite or obscured by other complications.

The investigations reported in this paper on the application of the Carr-Price ('26) color reaction for estimating vitamin A storage in livers were undertaken with the hope of making this technic an aid to diagnosis, particularly in suspected hypovitaminosis where historical, clinical, and pathological findings are not decisive.

## EXPERIMENTAL

*Vitamin A storage in the livers of turkeys and chickens fed various levels of dehydrated alfalfa leaf meal*

The birds used in this study were hatched and reared by the Division of Poultry Husbandry. They came from an investigation on the comparative pathology of avitaminosis-A



in chickens and turkeys, which will be reported elsewhere. As the present work was not outlined until near the close of the experiment, data regarding birds on low levels of vitamin A under 7 months of age were not available. The chickens were single comb White Leghorn females, while the turkeys were of the Bronze variety and about equally divided as to sex. The basal ration in the avitaminosis-A experiment was as follows:

	<i>Pounds</i>
Ground white corn,	25
Ground barley,	25
Ground wheat,	25
Fish meal,	10
Dried skim milk,	10
Bone meal,	3
Ground limestone,	2
Sodium chloride,	0.5

Vitamin A was supplied by adding freshly cut alfalfa or dehydrated alfalfa leaf meal containing approximately 130 gamma of carotene per gram in the proportions indicated in table 1. The mash was fed in hoppers to which the birds had access at all times. They were also exposed to direct sunlight for several hours daily.

The data on the liver storage of vitamin A presented in table 1 were obtained by quantitative estimation of the blue color produced by antimony trichloride reacting with chloroform solutions of the liver oils, a dilution procedure being used. The technic has been given in detail in another publication (Guilbert and Hart '34).

In each lot designated in table 1, the chickens and turkeys were kept in the same pens and fed from the same hoppers so that their feeding and environmental conditions were identical. The data show a direct correlation between the amount of dehydrated alfalfa leaf meal in the ration and the storage of vitamin A in the livers as represented by the color test. The chickens, though about 1 week younger than the turkeys when autopsied, showed greater storage of vitamin A than the turkeys on comparable rations.

Average growth curves of the various lots are presented in figure 1 which show that the growth of the turkeys was more retarded than that of the chickens fed comparable levels of dehydrated alfalfa leaf meal. The limitation of growth in the turkeys on the 2 per cent level (lot 4) was comparable to that of the chickens fed at the 1 per cent level (lot 5). There was heavy mortality among the turkeys and none among the chickens in these lots. All the turkeys on the 1 per cent level

TABLE 1

*Vitamin A storage in the livers of chickens and turkeys fed various levels of vitamin A from hatching to autopsy at 28 to 30 weeks of age*

LOT NO.	RATION	NUMBER OF LIVERS TESTED		AVERAGE NUMBER OF UNITS PER GRAM OF LIVER <sup>1</sup>	
		Chickens	Turkeys	Chickens	Turkeys
1	Basal ration + fresh alfalfa 20 weeks; 8 per cent D.A.M. <sup>2</sup> last 8 weeks	1	2	250	100
2	Basal ration + 8 per cent D.A.M.	4	3	270	65
3	Basal ration + 4 per cent D.A.M.	3	3	150	30
4	Basal ration + 2 per cent D.A.M.	4	4	36	1+
5	Basal ration + 1 per cent D.A.M.	4	..	4	...

<sup>1</sup> In our work a unit is defined as the amount of chromogen in 0.05 to 0.2 cc. of test solution which will give with 2 cc. of SbCl<sub>3</sub> reagent the faintest detectable blue color, using the technic outlined by Guilbert and Hart ('34). This amount of blue color is equivalent to that produced by 1 to 1.5 gamma of crystalline carotene (S. M. A. Corporation. M.P. 166° to 168°). The units reported here should not, therefore, be confused with blue units based upon the Lovebond tintometer.

<sup>2</sup> D.A.M. = dehydrated alfalfa leaf meal.

of leaf meal died before reaching 20 weeks of age, and symptoms of avitaminosis-A occurred among the turkeys even on the 4 per cent level. None of the chickens, on the other hand, manifested clinical evidence of vitamin A deficiency except for mild indications, toward the end of the experiment, in some of those on the 1 per cent level. Thus the growth and clinical data confirm the results of the color test.

The experiment was repeated with four additional lots of later hatched turkeys, omitting the 1 per cent level of de-

hydrated leaf meal. These birds did not do so well on the 2 per cent and 4 per cent levels of leaf meal as those in the

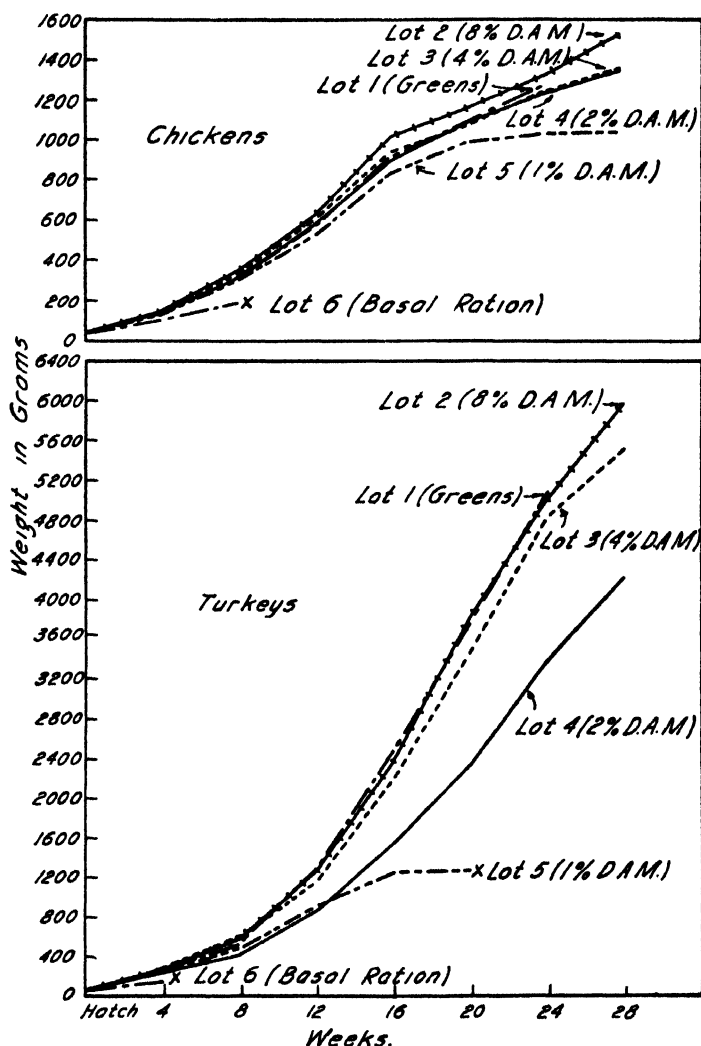


Fig. 1 Composite weight curves of turkeys and chickens fed various levels of vitamin A. (D.A.M. = dehydrated alfalfa leaf meal.)

first experiment, and the mortality was higher. Although the storage in the livers followed the trend shown in table 1 it was consistently lower.

*Correlation between vitamin A storage in the liver as determined by the color test and period of survival of birds from the same lot when placed on the basal ration.* At the time when the birds were autopsied to secure the data presented in table 1, three additional turkeys showing no visible evidence of deficiency were selected from each lot and placed on the basal ration. Three chickens from lot 4, and three from lot 5 were similarly treated. The birds in lots 3, 4 and 5 that were

TABLE 2

*Correlation between vitamin A storage in the livers and the survival period of penmates placed on the basal ration*

LOT NO.	ORIGINAL RATION	UNITS PER GRAM OF LIVER AT 30 WEEKS	TIME AFTER PLACING ON BASAL RATION		
			First clinical symptom (days)	First death (days)	Last death (days)
1 (turkeys)	Basal ration + green alfalfa	100	96	... <sup>1</sup>	140
2 (turkeys)	Basal ration + 8 per cent D.A.M. <sup>4</sup>	65	87	107 <sup>2</sup>	135
3 (turkeys)	Basal ration + 4 per cent D.A.M.	30	56	90	... <sup>3</sup>
4 (turkeys)	Basal ration + 2 per cent D.A.M.	1+	33	53	86
4 (chickens)	Basal ration + 2 per cent D.A.M.	36	56	60	85
5 (chickens)	Basal ration + 1 per cent D.A.M.	4	14	24	37

<sup>1</sup>Two birds killed to determine the blue value at the time of first clinical symptoms.

<sup>2</sup>One bird killed ninety-sixth day which probably would not have lived to the one hundred and seventh day.

<sup>3</sup>Remaining birds at 90 days were in advanced stages of deficiency and were autopsied.

<sup>4</sup>D.A.M. = dehydrated alfalfa leaf meal.

placed on the basal ration were in better condition than those autopsied at 30 weeks. Since the birds autopsied represented as nearly as possible an average, including the better birds as well as those in poor physical condition, their storage is not strictly comparable to that of the birds placed on the basal diet. The data are presented in table 2.

Table 2 shows a direct correlation between the blue values found in the livers of birds autopsied at 30 weeks and the survival period of penmates placed on the vitamin A deficient basal ration.

Birds autopsied upon showing the first clinical symptoms of vitamin A deficiency gave blue values for the liver extract varying from a trace to 2 units per gram. Birds in this condition usually survived 20 to 30 days. In lot 4, therefore, the turkeys placed on the basal ration evidently had greater storage than their penmates autopsied at 30 weeks of age. No blue color was obtained with antimony trichloride reagent from any of the liver extracts of numerous cases, both chickens and turkeys, that were killed in cachexia or died of vitamin A deficiency.

TABLE 3

*Comparison of vitamin A storage in the livers of normal turkeys of various ages*

AGE	NUMBER OF LIVERS TESTED	AVERAGE UNITS PER GRAM OF LIVERS	EXTREMES UNITS PER GRAM OF LIVERS	REMARKS
10 weeks	6	50	.....	Average of 2 composite samples from 3 males and 3 females
17 weeks	4	335	140- 400	Average of 4 individual determinations; 2 males and 2 females
21 weeks	4	620	500- 800	Average of 4 individual determinations; 2 males and 2 females
12-15 months	19	970	300-2000	Average of 8 individual and 3 composite samples. All females
2 years or older	11	2000	1300-3000	Average of 3 composite and 2 individual samples. All females

*Vitamin A storage in the livers of turkeys fed high levels of vitamin A*

The turkeys used for this phase of the studies were raised, from hatching to time of autopsy, on rations abundantly supplied with vitamin A. The mash contained 17 to 25 per cent yellow corn and 5 per cent dehydrated alfalfa leaf meal. In addition, the birds had access to all the fresh greens they would consume. For the first 4 weeks after hatching, fresh green lettuce was fed; from then on freshly cut and chopped alfalfa.

The variation of vitamin A storage in turkeys of different ages on this ration is shown in table 3.

The 10, 17 and 21 weeks old groups were hatched in 1933 from closely related parent stock. The 12 to 15 months old groups had completed the first laying period; the older birds, two or more laying seasons. As the data show, liver storage of vitamin A gradually increases during growth, reaching the highest values at maturity. This finding agrees with numerous data on other species.

Considerable variation existed in the liver storage of individuals within an age group. No correlation to explain it was found between liver storage and egg production or the functional state of the ovaries at the time of autopsy nor were sex differences noted.

Chickens of comparable ages were not available for comparison with the turkeys. Ten individual determinations were, however, made on the livers of 8 months old females fed essentially the same ration as the growing turkeys referred to in table 3. The average value found was 1150 units per gram. Two composite samples, each consisting of three livers from penmates, were taken at 12 months of age. These gave values of 4000 and 5000 units per gram, respectively. At 8 months the birds were practically mature and were producing eggs. Apparently the ration contained sufficient vitamin A not only for heavy production but also for significant storage, as judged by the values obtained for the penmates at 12 months of age.

The chickens at 8 months of age may be considered comparable in maturity to turkeys 12 to 15 months old. The liver storage at these ages again shows the tendency toward higher values in the chickens than in the turkeys fed similar rations.

#### *Individual variation in storage of vitamin A*

Attention has already been called to individual variation in the vitamin A liver storage of turkeys. Of the ten individual liver samples from 8 months' old chickens mentioned in the preceding paragraphs, four gave values varying from 1000 to 2500 units per gram; three gave values of 500 to 700

units; while two gave values of 100 units each and the remaining sample contained only 20 units per gram. This last value, at first thought to result from faulty technic, was checked by a second set of determinations on this liver. These birds were from the same source and from closely related stock, had received the same feeds, and were all producing eggs at the time of autopsy. (Trapnest records were not available.)

The variation in the liver storage of the normally fed birds, both chickens and turkeys, which had free access to green feed appears much greater than in the experimental lots where all the vitamin A carrying feeds were incorporated in the ration in a manner that made selection difficult. The results suggest that the variation in storage may be partly caused by individual differences in food habits. The variations found by the color test are comparable to those commonly noted in biological tests. Sherwood and Fraps ('32), for example, reported a variation of 35 to 199 days in the life of White Leghorn pullets after changing from an adequate to a vitamin A free diet. The average survival was 135 days.

A large error in expressing liver storage on the basis of a unit weight of liver tissue lies in the variation of the weight of the organ depending on the storage of glycogen and other products of digestion. The liver weights tend to become constant after 24 hours of fasting. The average weight of livers from twelve turkeys fasted 24 to 48 hours was 67 gm.; from seven non-fasted birds, 99 gm. The difference in fasted and non-fasted chickens was usually greater than in the turkeys. For example, the average liver weight of three chickens fasted 48 hours was 18 gm., as compared with 37 gm. for a like number of non-fasted birds. The livers of non-fasted chickens were commonly much lighter in color and more friable than those of fasted birds. The significance of the weight variation was not fully appreciated during the early part of this investigation. Fortunately, however, practically all our data are based upon liver samples from birds which had been fasted 24 hours or more before autopsy.

*Observations on the nature of the chromogenic substances in livers*

Chickens of the yellow-skinned breeds have more highly pigmented fat and liver extracts than turkeys of comparable maturity receiving the same feeds. One may therefore logically inquire whether the higher values found for chickens are partly caused by these pigments rather than by vitamin A.

Palmer ('22) has shown that the pigment of the body fat of chickens is largely xanthophyll and we have found this to

TABLE 4

*Distribution of total chromogenic value with antimony trichloride between the petroleum ether and the alcohol fractions of liver extracts*

SAMPLE	SPECIES	TREATMENT BEFORE AUTOPSY	TOTAL UNITS PER GRAM OF LIVER (DIRECT DETERMINATION)	PETROLEUM ETHER FRACTION—UNITS PER GRAM OF LIVER	85 PER CENT METHYL ALCOHOL FRACTION—UNITS PER GRAM OF LIVER
Composite of 3 livers	Chicken	Fasted 48 hours	5000	300	5000
Composite of 3 livers	Chicken	Not fasted	2000	400	1700
Composite of 4 livers	Turkey	Fasted 24 hours	600	50	500
No. 88	Sheep	Carotenoid—deficient ration	1000	200	800
No. 28	Sheep	Carotenoid—deficient ration	....	30	1000

be true also in turkeys. The pigments of several liver extracts have been separated by the phase method. The non-saponifiable matter was taken up in a small quantity of low-boiling, light petroleum and this was extracted by means of four to six treatments with 85 per cent methyl alcohol. The distribution of the total chromogenic value between the petroleum ether fraction containing the carotene and the alcohol fraction containing the xanthophyll is shown in table 4.

The non-saponifiable matter from each fraction obtained from the first sample shown in table 4 was dissolved in 10 cc.



of chloroform. Comparison of the depth of yellow color in a colorimeter gave nearly equal amounts in the carotene and the xanthophyll fractions, whereas most of the chromogenic value with antimony trichloride was found accompanying the xanthophyll.

The liver weights of the non-fasted chickens (referred to in table 4) were twice those of the fasted birds. If allowance is made for the dilution caused by storage of glycogen, etc., the blue values would become about 800 units and 3500 units per gram of liver for the petroleum ether and alcohol fractions, respectively. Although the carotene is significantly reduced, the conversion to vitamin A appears far from complete after 48 hours of fasting.

In some samples most of the pigment in the non-saponifiable matter of turkey livers was extracted by alcohol—a fact showing that the yellow color was largely due to xanthophyll. In others, however, there appeared to be about equal amounts of pigment in each fraction. Since strong blue color reactions develop in the alcohol fractions from the chicken livers at dilutions where only faint yellow color is discernible, and since xanthophyll gives less color with antimony trichloride than does carotene (Gillam, Heilbron, Morton, Bishop and Drummond, '33) it is evident that most of the blue color in the alcohol fraction is produced by vitamin A. This laboratory is not equipped to make spectroscopic determinations. In the Plant Physiology Laboratory at Berkeley the alcohol fraction of one sample gave with antimony trichloride a blue color having a very intense absorption band in the region of 610 m $\mu$ , while no perceptible band was observed in this region in the petroleum ether fraction. Several workers have, however, found vitamin A in both fractions; and the results obtained with the sheep livers shown in table 4 and with cod liver oil confirm these findings, as the non-saponifiable matter from these sources was practically colorless. A relatively large proportion of the total blue value obtained from chicken and turkey liver extracts may therefore be considered to come from vitamin A, particularly when the birds have been fasted.

## SUMMARY

A study of the Carr-Price reaction as a means for estimating liver storage of vitamin A in turkeys and chickens is reported.

A direct correlation was found between the liver storage (as expressed by the color test with antimony trichloride) and the level of vitamin A in the ration, the growth and mortality records, and the survival period of penmates when placed on the vitamin A deficient basal ration.

White Leghorn chickens were found to have greater storage of vitamin A than Bronze turkeys comparable as regards maturity and feeding history.

Large variations in liver storage were found among individuals from pens receiving the same feeds. It is suggested then, when selection is possible, individual variation in food habits may partly account for the variation in storage. Attention is called to similar variations in storage found in biological tests.

Turkeys receiving 8 per cent dehydrated alfalfa leaf meal showed a much lower liver storage of vitamin A than did turkeys having access to green feed in addition to vitamin A carrying feeds in their mash. Though the 8 per cent level of leaf meal sufficed for normal growth, the reserves were inadequate for protection against long periods of low vitamin A intake such as may occur on certain turkey ranges.

Data are presented on the correlation between vitamin A storage and age. Like other species, young growing turkeys had relatively little storage even on rations abundantly supplied with the vitamin. On such rations liver storage increased rapidly as percentage rate of growth decreased.

The blue value of liver samples taken at the time of the first clinical evidence of deficiency varied from a trace to 2 units per gram of liver. Birds in this condition survived from 20 to 30 days. Numerous tests on liver extracts from birds that died of avitaminosis-A or were killed in cachexia gave no blue color with antimony trichloride.

Though the intensity of the blue color reaction using the technic described by Guilbert and Hart ('34) has limitations from the standpoint of a precise quantitative method, the simplicity of the procedure and equipment renders it adaptable to general use. As shown by the data here presented, it may be a valuable aid to diagnosis and may also add materially to the information obtained by experiments on vitamin A requirements of fowls.

#### ACKNOWLEDGMENTS

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#### LITERATURE CITED

- CARR, F. H., AND E. A. PRICE 1926 Color reactions attributed to vitamin A. *Biochem. J.*, vol. 20, p. 497.
- GILLAM, A. E., I. M. HEILBRON, R. A. MORTON, G. BISHOP AND J. C. DRUMMOND 1933 Variations in the quality of butter, particularly in relation to vitamin A, carotene and xanthophyll content as influenced by feeding artificially dried grass to stall-fed cattle. *Biochem. J.*, vol. 27, p. 878.
- GUILBERT, H. R., AND G. H. HART 1934 Storage of vitamin A in cattle. *J. Nutrition*, vol. 8, p. 25.
- PALMER, L. S. 1922 Carotinoids and related pigments. The Chem. Catalogue Co., pp. 1-316. New York.
- SHERWOOD, R. M., AND G. S. FEAPS 1932 The quantities of vitamin A required by pullets for maintenance and for egg production. *Texas Agr. Exp. Sta. Bul.* 468, pp. 1-19.

# VARIATIONS IN THE POTENCY OF CERTAIN FOOD-STUFFS IN THE CURE OF DERMATITIS INDUCED IN RATS BY DIETARY EGG WHITE <sup>1</sup>

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It has been found that dietary egg white is capable of producing severe dermatitis and nutritive failure in both rats and chickens when it is incorporated in certain well supplemented rations,<sup>2</sup> even when the vitamin G content is in excess of the amount sufficient to insure growth and protection from dermatitis in ordinary diets. When the egg white is treated in one of various ways, or the concentration of certain potent foodstuffs in the diet is sufficiently high, however, dermatitis may be cured and nutritional well-being restored.<sup>2</sup>

Although the syndrome produced by means of these presumably adequate rations containing egg white is strikingly similar to that occurring on purified diets low in vitamin G, doubt that the protective factors involved in the two cases are identical has been occasioned by finding certain differences in distribution and resistance to extraction by solvents (Boas, '27; Parsons and Kelly, '33). The present experiments were undertaken to investigate the nature of the factor

<sup>1</sup> This work was aided in part by a grant from the University of Wisconsin Research Fund. A preliminary report of a part of this investigation was presented to the American Society of Biological Chemists at Cincinnati, April 11, 1933 (Parsons, H. T., J. G. Lease and E. Kelly, 1933, *J. Biol. Chem.*, vol. 100, p. lxxvii; *Proc. Am. Soc. Biol. Chem.*, 1933, vol. 8, p. lxxvii). Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The literature has been reviewed by Parsons ('31) and by Parsons and Kelly ('33).

or factors protective against dermatitis due to egg white. In this paper, only the general sources and properties of the factor as revealed by manipulations aimed at its isolation are reported.

#### EXPERIMENTAL

The characteristic syndrome due to dietary egg white was produced in albino and hooded rats on ration B as previously described (Parsons and Kelly, '33). When soreness of the lip was definitely established on this ration, a given foodstuff or preparation to be tested was fed in basal ration S, replacing part of the sucrose. This ration contained 40 per cent of Chinese dried egg white, 10 of wheat embryo, 4 of Osborne and Mendel ('19) salt mixture and 46 of sucrose with daily additions of 3 to 5 drops of cod liver oil. When the preparation to be tested lacked the vitamin B complex, 10 per cent of yeast was also substituted for an equivalent weight of sucrose in the basal ration (ration H). Without the addition of potent substances other than the amount of yeast and wheat embryo used, both rations B and H lead to decline and frequently to death within the 30-day test period following the establishment of the symptoms. The potency of the preparations tested was judged by the same criteria that have been used previously in determining the loss of toxicity in heated egg white (Parsons and Kelly, '33).

Unless otherwise stated, the various foodstuffs to be tested were fed after thorough cooking and drying.

Hemoglobin determinations were made by the Newcomer method with the use of a calibrated colored glass disk.<sup>3</sup>

#### RESULTS

*Occurrence of the potent factor.* No systematic investigation of foodstuffs as sources of the factor was attempted; the materials tested were those which gave promise of being rich in the factor or were otherwise especially suitable for investigation.

<sup>3</sup> We are indebted to Dr. C. A. Elvehjem, of the Department of Agricultural Chemistry of the University of Wisconsin, for calibrating our calorimeter.

Cooked pork liver and beef kidney proved to be about as effective as the cooked beef liver previously reported (Parsons, '31). Cooked pork kidney was one and one-half times as potent as beef liver, and ranks as the richest source of the factor protective against the dermatitis due to egg white which has thus far been discovered; a 5 per cent<sup>4</sup> concentration in basal ration S led to pronounced improvement in the dermatitis (table 1). Negative results with  $\frac{1}{2}$  of 1 per cent of adrenal glands (beef) indicated that the potency of the kidney tissue was not attributable to a possible contamination with adrenal tissue. Seven per cent of beef ovary, 7 and 30 per cent of beef heart, 5 per cent of pork heart, 7 and 30 per cent of beef spleen, and 5 per cent of pork spleen were entirely ineffective in improving the condition of the rats. Hemoglobin fed at 3 per cent, cooked beef blood at 7, and raw dried dog blood at 10 per cent were entirely ineffective in relieving the dermatitis due to egg white, in spite of the fact that they furnished much higher concentrations of hemoglobin than were present in the curative doses of liver or kidney used in our experiments. These results are not in harmony with the published statement of Bliss and Thomason ('30-'31) that dried whole dog blood, dried hemoglobin and crystalline hemin prepared from dog blood were effective in curing the pellagra-like manifestations which these authors produced in rats on a non-purified diet of natural foodstuffs in which raw egg white furnished the chief source of protein.

Five times the percentage of ferric citrate found in Osborne and Mendel's salt mixture, and 0.5 mg. per day of copper fed as copper sulfate, or these fed together were entirely ineffective for either protection or cure as was the ash of 20 per cent of beef liver. Furthermore, a study of the hemoglobin concentration<sup>5</sup> in the blood of the rats on egg white

<sup>4</sup> Percentages of this and other preparations are expressed on the air-dry basis.

<sup>5</sup> Credit is due to the following senior students in the Department of Home Economics for their assistance in making simultaneous hemoglobin determinations with one of the authors in many of the experiments: Gertrude Irwin, Catherine Johnson, Jeanette Lepp, Milada Prochaska and Norma Vesperman.

diets showed that although the average values tended to be somewhat lower than the average for rats on very good diets, they fell within the range considered normal for rats from 50 to 90 days of age (Williamson and Ets, '26) and did not

TABLE 1

*Comparative potency of certain foodstuffs and preparations in curing the dermatitis due to egg white*

FOODSTUFFS <sup>1</sup>	CONCENTRATION OF SUPPLEMENT WHICH IS				CONCENTRA- TION OF EGG WHITE IN RATION
	Very potent	Moderately potent	Slightly potent	Not potent	
	per cent	per cent	per cent	per cent	per cent
Raw beef liver				7, 10, 15	40
Raw pork liver			15	5	40
Raw beef kidney				5 <sup>a</sup> , 15 <sup>a</sup>	40
Raw pork kidney		10	7		40
Cooked fresh beef liver	10	7	5	3	40
Cooked fresh beef kidney			5 <sup>a</sup>	3	40
Cooked dried beef kidney		15 <sup>a</sup>			40
Cooked fresh pork liver			5		40
Cooked dried pork liver	15				40
Cooked fresh pork kidney	7	5		1, 3 <sup>a</sup>	40
Eli Lilly & Co. liver extract no. 343				10, 30	40
Liver residue from Eli Lilly & Co. extract		7			40
Wheat embryo	76				20
Wheat embryo		66			30
Yeast	50	40	30		40
Dried skim milk				60	40
Dried skim milk	80				20
Dried whole milk				50, 60	40
Dried whole milk			70		30
Egg yolk			60	40	40
Egg yolk		70			30
Raw beef liver, 37° 20 hours, then cooked	7		3		40
Raw beef liver, 23° 20 hours, then cooked	7				40
Raw beef liver, + N/50 HCl 37° 10 days		7			40
Raw beef liver, + N/50 HCl 37° 10 days then cooked	7				40
Raw beef liver, 25° 22 days then cooked		7			40

TABLE 1—Continued

FOODSTUFFS <sup>1</sup>	CONCENTRATION OF SUPPLEMENT WHICH IS				CONCENTRATION OF EGG WHITE IN RATION
	Very potent	Moderately potent	Slightly potent	Not potent	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Cooked pig kidney, moist 25°, 16 days		3	1		40
Raw fresh beef liver, 6 days at 100°, kept moist				7, 15	40
Raw fresh beef liver, 6 days at 100°, dry				7	40
Raw dried beef liver 3½ days at 100°, dry			7		40
Raw dried beef liver 6 days at 100°, dry		15			40
Cooked beef liver, dried, 3½ days at 100°, kept moist				7	40
Cooked beef liver, dried, 3½ days at 100°, dry				7	40
Cooked beef liver, dried, 6 days at 100°, kept moist				15	40
Cooked beef liver, dried, 6 days at 100°, dry				15	40
Raw pork kidney, dried, 3½ days at 100°, kept moist		7			40
Raw pork kidney, dried, 3½ days at 100°, dry			7		40
Cooked pork kidney, dried, 3½ days at 100°, dry	7				40
Raw fresh pork kidney, 6 days at 100°, dry		7			40
Raw pork kidney, dried, 6 days at 100°, dry			7		40
Cooked pork kidney, 6 days at 100°, kept moist	7				40
Cooked pork kidney, 6 days at 100°, dry				7	40

<sup>1</sup> Foodstuffs were fed dry; the weight given is on the air-dried basis.

<sup>2</sup> These groups had equalized food consumption.

decrease with the length of time on the egg white rations. For example, a group of nineteen rats showed an average hemoglobin concentration of 13.3 gm. per 100 cc. of blood (ranging from 10.6 gm. to 15.5 gm.) after 35 to 45 days on the



egg white diet, the period just preliminary to the appearance of sore lips and other manifestations of dermatitis. Also no correlation was found between the hemoglobin concentrations at weaning when the egg white diet was begun and the rate at which the symptoms appeared.

Since it seemed possible that a dehydration of the blood might be masking an actual anemia, blood solids were also determined in one series. Hardening and necrosis of the caudal tissues at the onset of dermatitis often interfered with taking blood samples from the tail, so that jugular blood samples were used in cases where the rats were sacrificed. The results showed that a group of nine rats, not exhibiting dermatitis, either because of protective foods fed or the short time on the ration, had an average concentration of 13.3 gm. of hemoglobin per 100 cc. of blood and 20.0 per cent of blood solids; thirteen rats with severe dermatitis, some in a critical condition, had an average concentration of 14.3 gm. of hemoglobin and 20.4 per cent of blood solids; and twelve rats, which had thoroughly recovered from a severe condition, had an average concentration of 12.9 gm. of hemoglobin and 20.6 per cent of blood solids.

In a few animals in which it was possible to obtain blood samples from the tail during an early period of recovery it was noted that hemoglobin values dropped markedly during the period of rapid gain in weight. For illustration, a rat in which severe dermatitis had been produced on ration B, had a blood hemoglobin concentration of 16.2 gm. per 100 cc. of blood. On changing the rat to a ration in which a favorable mixture of fresh egg white and fresh egg yolk was substituted for the Chinese dried egg white of the diet, a rapid cure ensued, with a gain in body weight of 6 gm. daily for 15 days and a drop in blood hemoglobin to a concentration of 12.4 gm. per 100 cc. during this time.

The failure of Eli Lilly & Co.<sup>\*</sup> liver extract no. 343 to lead to a cure of dermatitis was striking. Fed as either 10 or 30 per cent of ration S, this concentrate exhibited no potency

<sup>\*</sup> Kindly furnished by Eli Lilly & Co., Indianapolis.

whatever; the four rats on the dosage died before the expiration of the 30-day test period, with exaggerated symptoms of the disorder. These results are the more significant because of the high content of vitamin G in this liver extract. On the other hand, the solid liver residue<sup>6</sup> from the manufacture of the commercial liver extract was as potent in curing the dermatitis as cooked dried beef liver prepared in the laboratory (table 1). In accord with these results, certain other vitamin G-rich substances were relatively ineffective in curing the dermatitis due to egg white. It was necessary to include dried brewer's yeast<sup>7</sup> in at least as great a concentration as the egg white of the ration (i.e., 40 per cent) before distinct improvement was obtained. Wheat embryo, egg yolk<sup>8</sup> or dried milk could not be fed high enough with 40 per cent of egg white to lead to satisfactory cure. Only when the dried egg white of the ration was decreased to a concentration lower than 40 per cent, so that more of the supplementary food could be included and less toxicity was furnished by the egg white, could full recovery be secured (table 1). Freshly gathered mushrooms (*Coprinus atramentarius*) and watercress were cooked, dried and fed at a 10 per cent concentration (approximately the equivalent weight of the rest of the ration if they had been fed moist) without leading to any improvement in the condition of the rats.

Inasmuch as the most potent foodstuffs were rich in nucleoprotein, their effectiveness might possibly be attributable to this constituent. However, a sample of nucleoprotein prepared from beef liver and fed as high as 3 per cent—a concentration greater than that in which it occurred in rations

<sup>7</sup> From the Northwestern Yeast Company, Chicago.

<sup>8</sup> The results on egg yolk were confirmed and extended in a Bachelor of Science thesis by Arlette Caldwell ('31) written under the direction of Miss Dorothy Hussemann in the Department of Home Economics (unpublished data). In this study, dried egg yolk fed with dried egg white prepared from fresh eggs in the proportion of one-fourth egg yolk to one egg white did not prevent the onset of dermatitis when fed to weanling rats; but in the proportions found in whole egg (64 per cent of yolk to 36 per cent of white, dry basis) or as whole egg itself, it gave considerable protection as only doubtful or minor injury occurred during a 70-day feeding period.

containing potent supplements of liver<sup>9</sup>—failed to cause any improvement in the dermatitis. The residue from the nucleoprotein preparation, however, was approximately as potent as other cooked liver samples tested.

The possibility of loss of potency in liver tissue by various means was investigated. Beef liver and pork liver obtained immediately after the animals were slaughtered were of approximately the same potency as similar tissue procured from a packing house. Similarly, no loss of potency occurred in the fresh beef liver through such processes as the following: allowing the raw liver to stand for 10 days at 37° after the addition of N/50 HCl and chloroform to insure suitable conditions for autolysis according to the method of Herron and McEllroy ('32); or, without these additions, for 20 hours at 37°, or for 20 hours at room temperature, during which intervals extensive bacterial action took place. (See table 1 for full series.) The dried cooked product underwent only slow loss of potency through standing for long periods of time at room temperature, inasmuch as one such lot tested was found to have lost not more than one-third of its potency after a year's time.

It was conceivable that the apparent toxicity of egg white might in reality be a destructive action on the potency of the protective factor or factors in the mixed ration. Therefore, beef liver was tested which had been standing in the laboratory for 3 years incorporated in an egg white ration. It was fed at a 15 per cent concentration in ration S, to make allowance for the expected loss of activity at room temperature noted previously when dried liver was stored alone. The rapid recovery of the rats on this dosage gave evidence that no significant increase in the rate of destruction of the factor had been occasioned by contact with the egg white.

The potency of raw liver or kidney was distinctly increased by the heating of these products at water bath temperatures

<sup>9</sup> This was estimated from the 0.55 per cent yield of nucleoprotein obtained in this experiment, which was in good agreement with Wohlgemuth's ('02-'03) figures, i.e., 0.3 to 0.4 per cent of nucleoprotein from beef liver.

for 15 minutes or longer (table 1). Experiments with equalized food intakes confirmed this observation amply although the concentration of raw liver sufficient to furnish an adequate supply of the factor was not reached, because the food intake fell off as the concentration of raw liver or kidney was increased. From the data obtained, the potency appeared to be doubled or trebled by cooking. Increase in the activity was about equally well accomplished by cooking the fresh tissue or by drying and re-moistening it before cooking; but it was less satisfactorily accomplished by heating it dry at 100°, possibly because a certain amount of destruction accompanied the process.

Prolonged heating of liver, whether moist or dry, raw or previously cooked, resulted in pronounced destruction of the potent factor. In general, the same results were obtained with pork kidney. The data are presented in table 1. Heat appeared to be less destructive to the factor if the material were kept moist; other manipulations such as previous cooking or drying had an influence on the rate of destruction.

The process of boiling cooked beef liver with 10 per cent hydrochloric acid for 6 hours, with subsequent neutralization and drying, yielded a product in which no activity was demonstrated. Decreasing the time of boiling to 1 hour allowed the persistence of some activity; 1 hour's boiling with 5 per cent hydrochloric acid was only moderately destructive, about one-half to two-thirds of the potency being retained. The same extent of destruction resulted from a similar heat treatment of pork kidney with tenth normal sodium hydroxide. Treatment with this concentration of alkali in the cold for 4 days did not decrease the potency.

#### DISCUSSION

The results of these experiments offer conclusive evidence that the injury due to egg white is not in the nature of an amino acid deficiency, inasmuch as 30 per cent of dried beef heart or 60 per cent of dried milk in diets containing raw egg white, failed to effect a cure of the dermatitis. The pos-

sibility that an antienzyme might be involved is apparently also precluded. One possible hypothesis for an interrelationship of factors in a given diet, which has accounted for the damaging effects of a high content of lard and of iron compounds in certain rations, i.e., the destructive action of one ingredient upon another in a ration mixture, does not explain the present phenomena. If an immunological relationship shall be proved to be involved in the injury due to egg white in these experiments, it must function in a manner much more intricate than any known at present. The protection afforded by certain foodstuffs and the loss of this through certain agents, as demonstrated by these experiments suggests the action of a factor in the nature of a vitamin with the distinction that its action as investigated so far, appears to be to counteract a positive injury from egg white. The possible relationship of such a factor to the vitamin B complex will be discussed in a forthcoming paper.

#### SUMMARY

Cooked pork kidney is the richest source investigated, of the factor or factors which cure or prevent the dermatitis due to egg white, as it is necessary to include only one-sixth of the weight of the egg white present in a ration, for a cure. Cooked beef liver, pork liver and beef kidney are good sources, being effective if present in the ration in a concentration about one-fourth that of the egg white. One to three times the weight of the egg white, of dried yeast, dried egg yolk, wheat embryo or dried milk must be incorporated to be curative. There is relatively little or no potency in spleen, heart, ovary, adrenal, blood or hemoglobin. The activity resides in the solid liver residue left from the preparation of Eli Lilly & Co. liver extract no. 343, not in the extract itself. The potency of liver does not depend on its nucleoprotein fraction.

Cooking increases the activity of raw liver or kidney; autolysis or extensive bacterial action does not decrease it. Prolonged heating, i.e., 6 days at 100°C. is destructive, especially if the material is dry. Boiling with hydrochloric acid

for 1 hour at 5 or more per cent decreases the potency appreciably.

A low hemoglobin concentration is not a feature of the syndrome due to dietary egg white.

The injurious action of egg white apparently does not depend on the destruction of some dietary factor within the ration mixture.

#### LITERATURE CITED

- BLISS, S., AND M. L. THOMASON 1930-1931 Iron deficiency in the white rat and the white mouse. *Proc. Soc. Exp. Biol. and Med.*, vol. 28, p. 636.
- BOAS, M. A. 1927 The effect of desiccation upon the nutritive properties of egg-white. *Biochem. J.*, vol. 21, p. 712.
- CALDWELL, A. 1931 An experimental study of the possible protective properties of egg yolk when incorporated in diets rich in egg white. Thesis, Univ. Wisconsin.
- HERRON, W. F., AND W. S. McELLROY 1932 Autolyzed liver therapy in pernicious anemia. *Science*, vol. 76, p. 127.
- OSBORNE, T. B., AND L. B. MENDEL 1919 The nutritive value of the wheat kernel and its milling products. *J. Biol. Chem.*, vol. 37, p. 557.
- PARSONS, H. T. 1931 The physiological effects of diets rich in egg white. *J. Biol. Chem.*, vol. 90, p. 351.
- PARSONS, H. T., AND E. KELLY 1933 The effect of heating egg white on certain characteristic pellagra-like manifestations produced in rats by its dietary use. *Am. J. Physiol.*, vol. 104, p. 150.
- WILLIAMSON, C. S., AND H. N. ETS 1926 The effect of age on the hemoglobin of the rat. *Am. J. Physiol.*, vol. 77, p. 480.
- WOHLGEMUTH, J. 1902-1903 Ueber das Nucleoproteid der Leber. *Z. physiol. Chem.*, Bd. 37, S. 475.



# THE RELATION OF AVITAMINOSIS C TO BLOOD CLOTTING<sup>1</sup>

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Several well-established observations upon blood changes which are due to avitaminosis C and which might be correlated with blood clotting are those of Morikawa ('20), Ohata ('30, '32), Randoin and Micheaux ('32), Micheaux ('31), Mettier and Chew ('32), Gronchi ('30), and Diblicek and Kucera ('33).

Bearing more directly upon the problem are the following investigations. Stockman ('03) has recorded, that in scurvy the blood coagulates normally. MacRae ('08) agrees with him but quotes Sir A. E. Wright who says that scurvy "eventuates in a defect in blood coagulability." These conclusions were founded upon observations of human subjects. Working with guinea pigs, Findlay ('21) found that scurvy had no effect upon blood clotting. Kugelmass ('32) states that deficiency in the clotting function in hemophilic children appears not to be altered by the vitamin content of the diet, but does not say that vitamin deficiency might not affect blood clotting. Ohata ('32) has found that in scorbutic guinea pigs not only is the blood coagulation time prolonged, but that there is a diminution in the number of blood platelets and in the amount of fibrinogen, thrombin and cephalin present.

<sup>1</sup> From a dissertation presented to the faculty of the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Master of Science.

<sup>2</sup> Merrell Fellow in Biochemistry.



*It is quite possible that, in the older literature at least, there may have been some confusion of the avitaminoses A and C. For this reason, the present study of some of the blood changes, and particularly of the clotting time of the blood, was made upon animals whose diet contained all the essential factors with the sole exception of vitamin C.*

#### EXPERIMENTAL

*A. Diet.* Thirty-four healthy guinea pigs, ranging in weight from 156 to 480 gm. were fed a basal diet of hay, oats, soy bean flour and sodium chloride, the latter three substances in the proportion of 65:35:1. In addition to this, eight of these animals received 4 cc. of fresh, unheated orange juice per day. The twenty-six experimental animals were given the same amount of orange juice which had been heated to destroy the vitamin C.

The animals fed the vitamin C free diet developed typical scurvy. Three of these assumed unquestionable 'face-ache positions.' Two of the scorbutic animals were completely cured by fresh orange juice after 37 days of the scorbutic diet. The controls remained healthy and grew normally.

*B. Methods.* Coagulation time was measured by means of Biffi-Brook's coagulometer, the blood being taken from the ear-vein. When this was impossible in certain severe cases of scurvy the blood was taken by heart puncture and a correction factor (0.9) applied.

The first series of platelets counts was made by the method of Fonio ('12), the rest by the method of Rees and Ecker ('23).

The red blood cells were counted with a Thoma Zeiss hemocytometer.

Hemoglobin was estimated by means of the Dare hemoglobinometer.

#### RESULTS

The lengthening of the blood coagulation time was the first apparent symptom of disease in twenty-one of twenty-six

cases. The prolongation of the clotting time became quite marked in the period immediately preceding death. It varied from 10 to 220 per cent, and averaged 54 per cent. The albinos showed a longer initial clotting time than did the others (tables 1 and 2).

In the scorbutic guinea pigs a reduction of platelets was found which averaged 36 per cent and which was closely parallel to the reduction of red blood cells (tables 3, 4, 5 and 6).

TABLE 1

*Clotting time (in minutes). Group of eight guinea pigs on a scorbutic diet plus 4 cc. orange juice per day*

	3/2/33	3/8/33	3/14/33	3/20/33	3/26/33	4/1/33	4/7/33	4/13/33	4/19/33	4/25/33	PER CENT INCREASE
Maximum	3.50	3.25	3.50	3.50	3.50	3.50	3.25	3.50	3.25	3.25	+ 10
Minimum	2.50	2.50	2.50	2.50	2.75	2.50	2.25	2.25	2.25	2.25	— 18
Average	2.97	2.91	2.84	2.84	2.97	2.91	2.81	2.75	2.72	2.72	— 8

TABLE 2

*Clotting time (in minutes). Group of twenty-four guinea pigs on scorbutic diet*

	3/2/33	3/8/33	3/14/33	3/20/33	3/26/33	4/1/33	4/7/33	4/13/33	PER CENT INCREASE
Maximum	4.25	4.75	5.00	5.00	7.20	5.00	5.00	6.30	+ 220
Minimum	2.25	2.25	2.25	2.25	2.50	2.75	2.75	2.75	+ 10
Average	2.34	2.52	2.88	3.05	3.36	3.28	3.49	4.92	+ 54

A decrease in hemoglobin was detected in the blood of the scorbutic animals, but as the disease progressed the blood underwent a slight change in color which prohibited the continuation of this determination.

In attempted estimations of anticoagulant it was noticed that if blood from a scorbutic guinea pig is allowed to clot and is then centrifuged or given an opportunity to synerese the proportion of serum obtained is two to three times as great as that obtained from normal blood by the same process.

TABLE 3

*Platelet counts. Group of eight guinea pigs on a scorbutic diet plus 4 cc. orange juice per day*

	3/2/33	4/1/33	4/13/33	4/25/33	PER CENT DECREASE
Maximum	370,000	390,000	390,000	390,000	+ 11
Minimum	320,000	320,000	320,000	320,000	— 15
Average	345,000	360,000	355,000	353,000	— 2

TABLE 4

*Platelet counts. Group of twenty-four guinea pigs on a scorbutic diet*

	3/2/33	4/1/33	4/13/33	PER CENT DECREASE
Maximum	390,000	290,000	270,000	+ 54
Minimum	310,000	190,000	150,000	+ 22
Average	348,000	233,000	188,000	+ 36

TABLE 5

*Red blood cells. Group of eight guinea pigs on scorbutic diet plus 4 cc. orange juice per day*

	3/2/33	4/1/33	4/13/33	4/25/33	PER CENT DECREASE
Maximum	6,700,000	6,500,000	6,500,000	6,700,000	+ 11
Minimum	5,700,000	5,600,000	5,900,000	5,800,000	— 8
Average	6,100,000	6,200,000	6,100,000	6,100,000	— 1

TABLE 6

*Group of twenty-four guinea pigs on scorbutic diet*

	3/2/33	4/1/33	4/13/33	PER CENT DECREASE
Maximum	6,700,000	5,000,000	4,200,000	+ 47
Minimum	5,700,000	3,500,000	3,100,000	+ 14
Average	6,100,000	4,500,000	3,700,000	+ 28

### CONCLUSIONS

It was found that the blood of scorbutic guinea pigs has a longer clotting time, a smaller number of blood platelets and of red blood cells, and a smaller amount of hemoglobin than does the blood of normal guinea pigs. It was also found that a greater proportion of the blood of scorbutic guinea pigs separates as serum than is the case in the blood of the healthy animals.

These changes in the blood begin before any other of the usual symptoms of scurvy appear, and it is possible that incipient scurvy in guinea pigs may thus be recognized from the blood changes before it is possible to detect it in other ways.

Whether in human beings these blood changes occur early in the disease has not been investigated.

These changes were found not to take place in young female albino rabbits in twice the time required for guinea pigs to die of scurvy when fed the same diet.

I wish to express my gratitude to Prof. A. P. Mathews for his helpful criticisms and suggestions.

#### LITERATURE CITED

- DIBLICEK, B., AND C. KUCERA 1933 L'image sanguine des cobayes scorbutiques et des convalescents. *Compt. rend.*, T. 113, p. 162.
- FINDLAY, G. M. 1921 The blood and blood vessels in guinea pig scurvy. *J. Path. Bact.*, vol. 24, p. 446.
- FONIO, A. 1912 Über ein neues Verfahren der Blutplättchenzählung. *Deutsch. Zeit. f. Chiur.*, Bd. 117, S. 176.
- GRONCHI, V. 1930 Resistance of red blood corpuscles to the hemolytic action of lysocithin in experimental scurvy. *Boll. soc. ital. biol. sper.*, vol. 5, pp. 637-638.
- KUGELMASS, I. 1932 Mechanism of hemophilia in infancy and in childhood. *Am. J. Diseases of Children*, vol. 44, pp. 50-68.
- MACRAE, D. M. 1908 Notes on scurvy in South Africa. *Lancet*, vol. 1, p. 1839.
- METTIER, S., AND W. CHEW 1932 Anemia of scurvy. Effect of vitamin C diet on blood formation in experimental scurvy of guinea pigs. *J. Exp. Med.*, vol. 55, pp. 971-979.
- MICHEAUX, A. 1931 Chlorine in experimental scurvy. *Bull. soc. hyg. aliment*, vol. 19, pp. 117-131.
- MORIKAWA, Y. 1920 Adrenal changes in experimental scurvy. *Endocrinology*, vol. 4, p. 615.
- OHATA, S. 1930 Studies in experimental scurvy. X. Adrenaline content of the suprarenals and the amount of adrenaline-like substance in the serum of guinea pigs fed on a vitamin-C-free diet. *J. Biochem. (Japan)*, vol. 12, pp. 419-427.
- 1932 Studies in experimental scurvy. XIV. The fatty acid and lipide content of the blood of guinea pigs on a vitamin-C-free diet. *Ibid.*, vol. 16, pp. 191-206.
- 1932 Studies in experimental scurvy. XV. Changes in the blood of guinea pigs fed on a vitamin-C-free diet. *Ibid.*, vol. 16, pp. 207-215.

- RANDOIN, L., AND A. MICHEAUX 1932 Variations, during the course of acute experimental scurvy, of the quantities of sodium and potassium eliminated by the kidneys and present in the blood serum and whole blood. *Compt. rend.*, T. 194, pp. 565-567.
- REES, H., AND E. ECKER 1923 An improved method for counting blood platelets. *J. Am. Med. Assoc.*, vol. 80, pp. 621-622.
- STOCKMAN 1903 Compare Gibson's System of Medicine, 1st ed., vol. 2, p. 21.

# THE DEVELOPMENT OF XEROPHTHALMIA AND THE KERATINIZATION OF EPITHELIAL TISSUE ON WITHDRAWAL OF VITAMIN A FROM THE DIET OF THE MONKEY (*MACACUS RHESUS*), GUINEA PIG, RABBIT, AND ADULT ALBINO RAT <sup>1 2</sup>

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SIX TEXT FIGURES AND FOUR PLATES (TWENTY-TWO FIGURES)

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In man (Wilson and DuBois, '23), in the albino rat (Wolbach and Howe, '25), and in the albino mouse (Wolfe and Salter, '31), a specific effect of vitamin A deficiency has been found in the development of an eye disease usually described as keratomalacia in man and xerophthalmia or ophthalmia in animals, and in a change in the epithelial structures resulting in the substitution of stratified keratinizing epithelium for normal epithelium in the respiratory tract, alimentary tract, genito-urinary tract, eyes, and para-ocular glands. It was observed by Daniels et al. ('23) that rats afflicted with xerophthalmia often showed suppurations in the nasal cavities and sinuses. When there has been a lack of vitamin A in the diet, xerophthalmia has been observed in the rabbit (Nelson and Lamb, '20), the chicken (Beach, '23), the dog (Steenbock et al., '21; Stimson and Headley, '33), and in cattle (Hart et al., '33). The cattle observed by these last-named authors showed not only xerophthalmia with severe corneal involve-

<sup>1</sup> Conducted under a grant from the Commonwealth Fund of New York.

<sup>2</sup> The observations reported in this paper were made on vitamin A depleted animals used in a study of the nutritional aspect of trachoma which will be reported in another paper.

ment but also lung involvement with difficult respiration, emaciation, and severe diarrhea. Analysis of liver samples of the cattle dead from vitamin A lack in the diet showed no vitamin A in the liver.

Clouding of the cornea but no typical conjunctivitis was observed by Boock and Trevan in the guinea pig ('22). Wolbach and Howe were unable to demonstrate the eye disease in guinea pigs but they did find extensive keratinization of the epithelial tissues of the uterus, the ducts of the submaxillary glands, the trachea, and the bladder ('28).

Xerophthalmia was reported by Saiki ('29) in two very small monkeys after 30 and 47 days, respectively, on a diet free from vitamin A (Saiki, '29). Turner and Loew ('31) fed the Saiki diet to monkeys but failed to produce xerophthalmia. Nor did their monkeys show any signs of keratinization of the epithelial tissues studied. The animals succumbed after 1 to 10 months on the diet with a loss of weight, loss of appetite, and gastro-intestinal symptoms. Post-mortem examination showed evidence of intestinal inflammation, marked enteritis, and dilatation of the stomach. The upper respiratory tract, nasal cavities, and middle ear were free from suppuration in all of their deficient animals. Turner and Loew also fed the same vitamin A low diet used by McCarrison ('20) but failed, as McCarrison had done, in showing any specific results characteristic of the deficiency.

Tilden and Miller ('30) again failed to produce xerophthalmia in monkeys even after 312 days on a ration containing only six to twelve units of vitamin A daily. There was no sinusitis or mastoiditis but in nine out of eleven monkeys keratinization occurred in the epithelial tissue of the seminal vesicle, the ureter, the bladder, or at the gastroesophageal junction. The animals reported by Tilden and Miller lived from 80 to 312 days on the vitamin A-low diet. In all of the monkeys studied there was a loss of weight followed by a loss of appetite and finally by severe colitis and death.

It has been generally concluded from these recent observations of Tilden and Miller and Turner and Loew that vitamin

A depletion in the monkey does not lead to the eye disease, xerophthalmia, but rather to a gastro-intestinal pathology. Observations made in our laboratory, however, indicate that a monkey depleted of vitamin A may react in the same characteristic manner as does the rat deprived of this vitamin. While xerophthalmia developed in only one of the twenty-seven monkeys under observation, this animal was the only one that lived longer than the monkeys reported by previous investigators. This animal remained in fairly good health for almost a year without showing severe colitis or loss of appetite and after 13 months on the diet showed keratomalacia of both eyes. The specific effect of the vitamin A deficiency was manifested in the substitution of stratified keratinizing epithelium for normal epithelium in some of the epithelial structures. This specific effect in addition to the development of xerophthalmia was also observed in vitamin A depleted guinea pigs, rabbits, and rats under observation in this investigation.

#### EXPERIMENTS WITH MONKEYS

Young monkeys, *Macacus rhesus*, weighing from 1 to 2 kg. each, were housed in individual heavy metal mesh cages with wire bottoms of large enough mesh to allow feces to fall through. The drinking cups of 500-cc. capacity were of monel metal and built into the door of the cage. The removable pans below the wire bottoms were also of monel metal. The cages and pans were cleaned three times weekly and the pans were sometimes cleaned oftener. Sawdust or shavings were kept in the pans.

The monkeys received food which was very low in vitamin A. The diet included a combination of rolled oats, vitamin A-free casein, cornstarch, sugar, hydrogenated vegetable oil<sup>3</sup>, egg white, Osborne-Mendel salt mixture, sodium chloride, and water mixed and baked into a cookie which was readily eaten by the monkeys. This mixture, called monkey 'cookie,' made according to the formula given in table 1, furnished most of

<sup>3</sup> Crisco.



the protein, carbohydrate, fat, minerals, and vitamins B, G, and E of the diet. The diet also included tomato serum,<sup>4</sup> a clear yellow juice which was shown by the writer ('30, unpublished data) and by Steenbock and Schrader ('31) to be almost lacking in vitamin A. It was also shown by the writer ('30, unpublished data) to be an excellent source of vitamin C. The tomato serum was used especially for its vitamin C contribution to the diet but it also added vitamins B and G, minerals, and carbohydrate to the daily food intake. From 3 to 4 ounces of tomato serum were given daily with the water. The

TABLE 1  
*Formula for monkey 'cookie'*

	<i>Gm.</i>	<i>Per cent</i>
Casein (alcohol extracted)	75	5
Oats, rolled	675	45
Cane sugar	375	25
Sodium chloride	15	1
Salt mixture (Osborne-Mendel)	60	4
Cornstarch	150	10
Hydrogenated vegetable oil <sup>1</sup>	150	10
Egg white	500	
Water	500	

After the above dry components were mixed thoroughly the hydrogenated vegetable oil<sup>1</sup> was added and the whole mixture combined with the egg white and water. It was then spread about  $\frac{1}{4}$ -inch thick on flat baking pans and baked for 3 to 4 hours in a moderately hot oven, when it was cut into strips about  $1 \times 3$  inches in size and baked for an hour or more longer to further dry the cookies.

<sup>1</sup> Crisco.

diet also included 6 drops daily of Viosterol, 250 D, for vitamin D. This was given in the cup with the tomato serum and water.

A weighed amount of monkey 'cookie' was given to each animal daily for the first 4 months of the feeding. Considerable of the food, however, was lost through the wire mesh bottom of the cage and therefore for the remainder of the experiment, monkey 'cookie' was given ad libitum. It was estimated that the animals ate daily of the 'cookie' and tomato serum enough food so that their average daily intake

<sup>4</sup> Tomato serum was purchased from the Welch Grape Juice Company.

was approximately 140 calories and 4.5 gm. of protein per kilogram body weight. The calories were distributed as shown in table 2.

TABLE 2

*Average distribution of calories in the foods eaten daily by the monkeys  
(This includes the monkey 'cookie,' tomato serum, and Viosterol)*

	Per cent
Protein	13
Carbohydrate	60
Fat	27

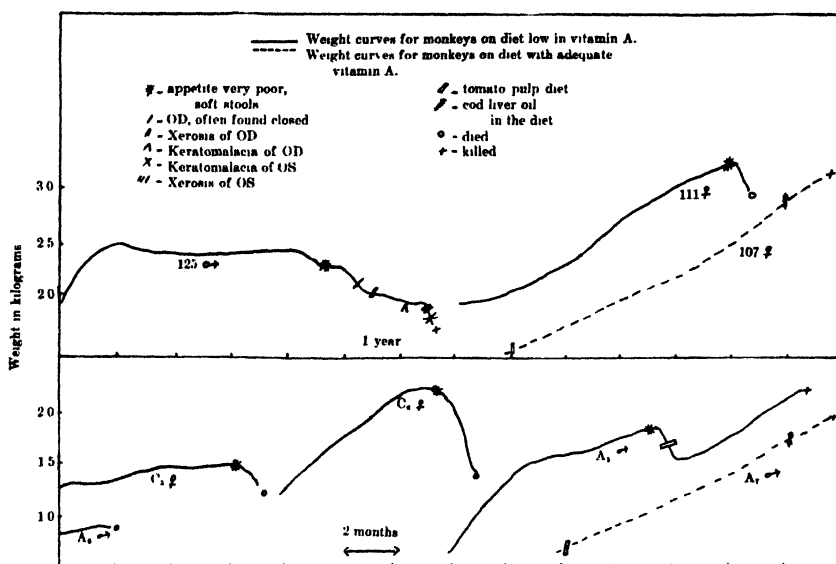


Chart A Weight curves of monkeys with and without vitamin A in the diet.

The monkeys were usually weighed every week and record made of the appetite and condition of the stools. If the appetite was below normal or the stools were soft, 5 to 10 gm. of dried yeast<sup>5</sup> were given daily for a few days or until the appetite and stools were again normal. The first effect of the yeast was to produce diarrhea which soon ceased but would always reappear if the yeast was continued for too long a period.

<sup>5</sup> Northwestern.

Some of the monkeys were in a very healthy condition when the feeding was begun but a few of them were very thin and unkempt in appearance when received. Of the 27 animals obtained for the study, 2 died from tuberculosis, 6 lived less than a month on the diet, 1 lived for 2 months, 4 lived for 3 months, 1 lived 4 months, 6 lived from 6 to 7 months, 1 lived from 9 to 10 months, and 1 monkey, 125, was killed after 13 months on the diet. Six of the depleted animals were fed tomato pulp for vitamin A after loss of appetite and loss of



Fig. 1 Monkey 125, showing xerophthalmia. The cornea of the right eye had perforated. The left eye is beginning to show keratomalacia.

weight occurred. Autopsy was made of all of the animals and histological examination was made of some of the tissues of monkeys 125, 111, S<sub>4</sub>, and C<sub>5</sub>. The livers of monkeys 125, 111, C<sub>4</sub>, C<sub>5</sub>, A<sub>8</sub>, and of a normal control were fed to vitamin A depleted rats to test for the presence of vitamin A.

Chart A shows typical weight curves of monkeys fed the vitamin A-low diet. Of greatest interest is the record of monkey 125. He was one of the first two monkeys given the diet in October, 1931. Both monkeys gained weight rather rapidly for a time and for 8 months were very healthy in

appearance. The fur then began to look slightly ragged and the appetites were none too good. Yeast was given daily for a week and the appetites improved but in June one of the monkeys died and at autopsy tubercles were found. To try to save monkey 125 so that the lack of vitamin A in the diet could be studied, this animal's cage was moved for the summer to the roof in the sunshine, and the daily intake of tomato serum was doubled. His appetite remained good and the stools were fairly well formed until late in July when the appetite was poor again and there was mild diarrhea. When yeast was again given the appetite improved as also did the stools but the weight was decreasing. Tomato serum was offered ad libitum and sometimes as much as 16 ounces were ingested daily. During the last of August, 10 months after the feeding was started, the right eye of this monkey was often found closed and he avoided looking at the light. The teeth began to loosen and fall out, but before the monkey was killed new teeth came in to replace the lost ones. Before the twelfth month on the diet, the right eye showed definite xerosis, the cornea was wrinkled, and there were Bitot's spots. The left eye was at times found completely closed. A corneal ulcer soon developed on the right eye and perforated. During the thirteenth month on the diet, the left eye showed the same changes which had occurred in the right eye, and as soon as the cornea became opaque, the animal was killed. Figure 1 shows a picture of the monkey after 13 months on the diet. He was somewhat inactive, lean and emaciated, and the hairy coat was very rough. There was no discharge from the nose and no severe diarrhea, although the stools were soft. There had been considerable loss of weight, from 2.5 kg. in June to 1.79 kg. in November. The autopsy and study of the animal tissues with the exception of the eyes, nose, and ears were made by Dr. Howard A. McCordock, of the Department of Pathology, Washington University Medical School.

Gross postmortem changes were not unusual except for an almost complete disappearance of body fat and an opacity in the pelvis of the kidneys. There were no ulcers in the intestine. The tongue gland, sinuses, and mastoids were free from pus. There was no vitamin A in the liver. Histological study revealed no abnormalities of the gastro-intestinal tract but in the eyes, ears, sinuses, salivary glands, and kidneys there was significant epithelial metaplasia. Figures 2 to 13 show the changes in these structures. For comparison the maxillary sinus of a normal well-fed monkey is shown in figure 14 and a normal temporal bone is pictured in figure 15.

Histological examination of the preparations of the eyeballs and eyelids of monkey 125 was made by Dr. Harvey D. Lamb, of the Department of Ophthalmology, Washington University Medical School. His report follows:

*Right eyeball.* The peripheral part of the cornea was thickened by edema and presented considerable proliferation of corneal corpuscles and infiltration with small lymphocytes and plasma cells in the anterior half of the corneal thickness. Bowman's membrane was entirely destroyed and many capillaries were present between the corneal lamellae in the anterior half of the cornea. Over the corneal center, practically the entire corneal thickness had been destroyed and anteriorly, a thin layer of cicatricial tissue was present. The large destroyed portion of the cornea was occupied by swollen prolapsed pupillary portions of the iris and a very thick cellular mass filling the pupillary space; the latter consisted of dense numbers of small lymphocytes, plasma cells, young fibroblasts and proliferated pigmented epithelial cells from the pigmented iris epithelium. Over almost the entire posterior surface of the iris, the pigmented iris epithelium showed an intense proliferation and the formation of pigmented spindle cells, probably to become fibroblasts. This proliferated epithelial tissue was moderately and diffusely infiltrated with plasma cells. The iris was edematous and showed a small greatly varying infiltration with plasma cells.

A perforation had occurred at about the corneal center and corneal epithelium had proliferated deeply along the wound edges. The lens-capsule had been ruptured and the lens almost altogether absorbed; in places dense numbers of pus cells were present within the lens capsule and in other places thin layers of proliferated lens epithelium.

The anterior epithelium of the cornea covered its entire surface; it was generally considerably thicker than normal and on its posterior surface showed the formation of papillae. An advanced degree of xerosis was present in the corneal epithelium with generally a thick keratinized layer, keratohyaline grains and much desquamation. The basal cells in places presented edema of the cytoplasm; all but the more superficial layers of cells showed thin layers of edema between the individual cells.

*Left eyeball.* The cornea was uniformly thickened by edematous fluid. At and near the corneal margin, numerous capillaries were present in the anterior half of the corneal thickness, extending for a short distance into the cornea. In the same locality proliferation of the corneal corpuscles was moderate in degree.

The iris-angle contained a small group of dense numbers of pus cells.

The corneal epithelium in many places showed a flattening of all the cells except those in the basal layer; superficial layers of flattened cells were dried and desquamating without a true keratinization being noted; the basal cylinder cells were slightly edematous and all but the superficial dried cells showed generally a thin vacuolar ring about the nucleus.

*Bulbar conjunctiva of both eyeballs.* An advanced degree of xerosis of the covering epithelium was present with a thick keratinized layer, keratohyaline grains and much desquamation. The basal epithelial cells, in places, presented cystic degeneration of their cytoplasm.

*Palpebral conjunctivae.* In all of the eyelids, generally, intensive xerotic changes were noted in the covering epithelium. Thick layers of keratohyaline grains and the desquamation of thick keratinized layers were common. The covering epithelium was generally much thicker than normal with several midlying layers of polyhedral-shaped cells. Thin layers of edematous fluid were present in many places between all but the superficial flattened epithelial cells. In only a few places, the cytoplasm of the deeper lying cells were edematous. The lower part of the lower right eyelid was densely infiltrated throughout its thickness by pus cells.

Histological examination of the preparations of the sinuses and ears was made by Dr. Wm. F. Wenner, of the Department of Otolaryngology, Washington University Medical School. His report follows:

The mucous membrane lining of the maxillary sinuses and the mucous membrane of the turbinates and the nasal septum of the monkey showed marked degree of metaplasia and keratinization of the epithelium with round cell infiltration in the basement membrane. The lining membrane of the bulla of the ear was very hyperemic. The blood vessels were greatly dilated and filled with blood. The compact bones immediately underlying the membrane show evidence of decalcification. There was no evidence of cellular resorption of bone. There were no osteoclasts present.

The other monkeys on the same vitamin A-low diet lived only from 1 to 10 months before loss of appetite and loss of weight occurred. The time of year and facilities were such that it was impossible to keep them outdoors. Of all the other animals, monkey 111 remained in the best condition for the longest time. Her death, as shown in chart A, followed almost immediately after the first indications of poor appetite, soft stools, and weight loss. She always looked very healthy. She never had diarrhea. Autopsy showed a normal animal. Histological examination of the tissues also showed a perfectly normal animal in all respects but one. The epithelium of the conjunctiva of the right eyelids showed fairly extensive metaplasia from stratified columnar to stratified squamous cell epithelium but there was no keratinization. There was no vitamin A in the liver.

Monkey A<sub>8</sub> weighed less than 1 kg. when the experiment was begun and lived only 2 months on the deficient diet. The appetite was always poor. Autopsy showed a small normal animal with very little fat. There was as much vitamin A in the liver of this animal as in the liver of the normal control. Another of the monkeys, however, that died after only 3 months on the diet, showed no vitamin A in the liver.

As chart A shows, monkeys C<sub>4</sub> and C<sub>5</sub> lived for almost 7 months on the deficient diet. C<sub>5</sub> gained very little weight but C<sub>4</sub> almost doubled her weight before the loss of appetite and loss of weight occurred. C<sub>4</sub> was typical of another monkey, T<sub>3</sub>, the only two monkeys studied showing complete loss of appetite, severe diarrhea, and abscesses on the face. Intes-

tinal ulcers were found at autopsy both in C<sub>4</sub> and in C<sub>5</sub>. Histological examination showed normal eyes, kidney, and salivary glands. The uterus of C<sub>4</sub> was filled with pus with bacteria present. There was inflammation of the intestinal mucosa. The large intestine of C<sub>5</sub> showed subacute colitis with an acute ulcer. There was oedema of the submucosa. Since this animal never gained much weight, it is possible that she died from a cause other than vitamin A deficiency for both C<sub>5</sub> and C<sub>4</sub> were extremely emaciated and looked sick before death. There was no vitamin A in the liver of either of these animals.

Monkey A<sub>5</sub> was typical of a group of six animals who were fed varying amounts of tomato pulp after they began to show loss of weight on the deficient diet. A<sub>5</sub> was given 4 ounces of whole tomato daily, considerably more than was fed to the other animals, and she made the best recovery. After 4 months of ingestion of vitamin A containing food, monkey A<sub>5</sub> looked normal again.

Although only one of the twenty-seven monkeys on the vitamin A-low diet showed xerophthalmia and the histological changes characteristic of vitamin A deficiency, it can be concluded that the monkey (*Macacus rhesus*) is susceptible to the same characteristic changes due to vitamin A deficiency that have been hitherto demonstrated in man and in the albino rat. Since monkey 125 showed keratinization of the epithelial tissues of the eyes, the sinuses, the kidneys, and the salivary glands after 13 months on the vitamin A-low diet and still showed a normal gastro-intestinal tract with no ulcerative colitis and had had no severe diarrhea, it seems possible that in the monkeys of Tilden and Miller and Turner and Loew and in the three monkeys here reported, colitis was due only indirectly or else not due to the deficiency of vitamin A. The diets used by the other investigators may have been more irritating to the intestinal mucosa than the diet fed to our monkeys. We found that more than 5 per cent casein and even a daily 5 gm. of yeast caused diarrhea. Also since our source of vitamin C, tomato serum, furnished almost no vita-



min A, it was possible to feed an unlimited amount of this food. With healthy monkeys to feed and a suitable environment with sunshine and fresh air it is probable that other monkeys could live long enough on a suitable vitamin A deficient diet to show the specific keratinizing effect with no other changes.

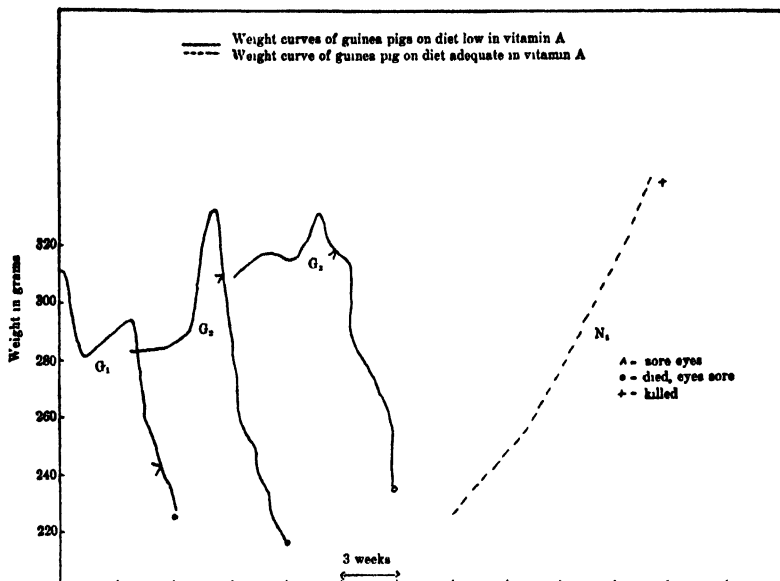


Chart B Weight curves of guinea pigs on diets with and without vitamin A.

#### EXPERIMENTS WITH GUINEA PIGS

Young guinea pigs weighing about 300 gm. each were housed in metal mesh cages similar to those usually used for rat breeding. The animals were offered the same vitamin A-low diet given to the monkeys. Although the guinea pigs ingested 3 to 4 ounces of tomato serum daily, their intake of monkey 'cookie' was very small. The three guinea pigs thus fed died in less than a month with no eye lesions. Three other animals were then offered the same diet with whole oats ad libitum. These animals, as chart B shows, lived for 6 to 9 weeks. During the last 2 or 3 weeks they lost weight very rapidly and developed eye lesions. The lids were dry

and scaly and the corneas were usually slightly clouded. The fur became rough and unkempt. The animals were very thin and emaciated. The stools of  $G_3$  were bloody during the last 2 days. All of the animals shivered for 1 or 2 days before death. Histological study was made only of the eyes of these animals. A report of the microscopic findings of the eyes of guinea pig  $G_3$ , from Dr. Harvey D. Lamb, follows:

*Eyeballs.* The anterior epithelium of the cornea contained two to three more superficial layers of flattened epithelial cells than are present in normal eyes. Over the cornea the two or three superficial layers of flattened cells were becoming keratinized and desquamating; desquamation of a single layer or of two layers together can be seen. In places, the basal layers showed considerable cystic degeneration in the cytoplasm of the cells.

*Bulbar conjunctiva.* Instead of the multilayered cylindrical celled epithelium, there were present varying degrees of change to polyhedral and flattened cells in all the layers with the exception of the basal cylindrical celled layer; in places, all the layers except the basal one were changed to layers of flattened cells. The nuclei of the polyhedral and flattened cells generally showed cystic degeneration and the condensation of the chromatin into a few coarse particles. In a few places two to three superficial layers of flattened cells have become hyaline-like and dark staining. In many places, single or two superficial layers together were becoming dry and keratinized and desquamating from the surface. In most places, therefore, keratinization of the superficial flattened cells is not preceded by hyalinization of several layers together. No keratohyaline grains are seen.

*Palpebral conjunctiva.* In all the four eyelids, there was present the same change in varying degree from a multilayered cylindrical celled epithelium to layers of polyhedral or flattened cells with the exception of the basal cylindrical celled epithelium. Many of the nuclei of the polyhedral and flattened cells showed cystic degeneration and a concentration of the chromatin into fewer and larger granules than is normal. In many places one or two layers together of superficial flattened cells were becoming keratinized and desquamating. The condition appears to be of about the same degree in the upper as in the lower eyelids.

Figures 16 and 17 show keratinization of the epithelial tissue of the lid and cornea of guinea pig  $G_3$ .

## EXPERIMENTS WITH RABBITS

Young rabbits, about 3 months old, and weighing 1.5 to 2.0 kg., were offered the same vitamin A-low diet given to the monkeys but, as with the guinea pigs, it was found that they soon died unless oats were also given. Of twelve rabbits fed the deficient diet, ten developed sore eyes in from 2 to 5 months. The other two animals died in less than 3 months before eye lesions had appeared. The rabbits gained in

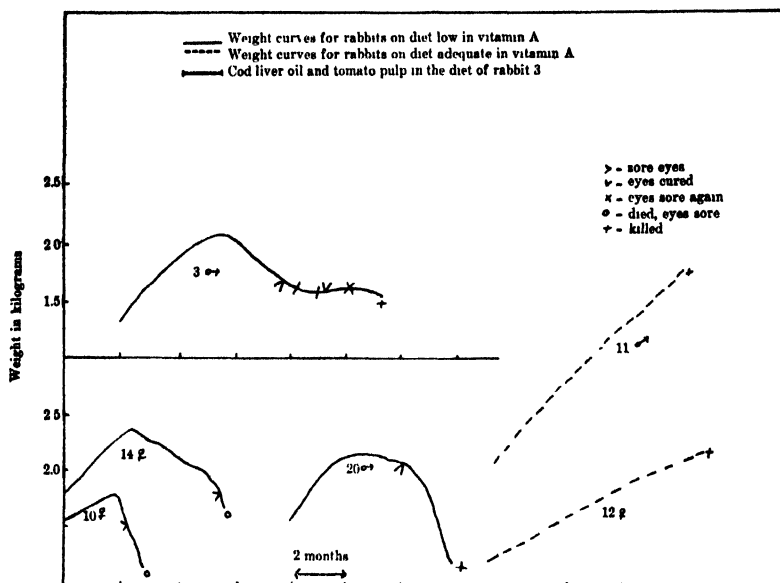


Chart C Weight curves of rabbits on diets with and without vitamin A.

weight, as shown in chart C, for a period of 2 to 4 months and appeared very normal and healthy. Usually the animals lost weight, sneezed, and had a slight discharge from the nose at about the time the lids became dry and scaly. In most of the rabbits the cornea became opaque before the animal died. When tomato pulp and cod liver oil were given to rabbit 3, the weight loss ceased and the eyes cleared, but when the vitamin A was discontinued, as is shown in chart 3, the eyes became sore again. Bitot's spots appeared in the eyes of all of the rabbits. The eyes of all of the rabbits and the

sinuses and ears of two of them were studied histologically. There were usually either keratomalacia or incipient keratomalacia of both eyes and advanced xerosis of the epithelium of the cornea, the bulbar conjunctivae, and the palpebral conjunctivae. Keratohyaline grains were noted in the bulbar conjunctiva of the left eye and corneal ulceration in the right eye of rabbit 3. Pus was found present in both eyes of all of the rabbits studied.

The mucous membrane lining of the maxillary sinuses and the mucous membrane of the turbinates of the nasal septum of the vitamin A-depleted rabbits showed a marked degree of metaplasia and keratinization of the epithelium with round cell infiltration in the basement membrane. The lining membrane of the bulla of the ear was very hyperemic. The blood vessels were greatly dilated and filled with blood. The compact bones immediately underlying the membrane showed evidence of decalcification. There was no evidence of cellular resorption of bone. There were no osteoclasts present.

The changes noted in the eyes, sinuses, and ears are shown in figures 18 to 22. Figure 23 shows the temporal bone of a normal rabbit contributed from another experiment.

Six rabbits weighing 0.8 kg. to 2.5 kg. were fed the control diet with adequate vitamin A. At first the rabbits ate very little tomato pulp, so both tomato pulp and cod liver oil were given. In about 2 months on such a diet, these control animals began to lose their fur and show stiffness in the legs. One very small rabbit weighing less than a kilogram became very crippled and lost practically all of the fur. It was suspected that cod liver oil might be responsible for this unusual condition in the rabbits and therefore it was omitted from the diet. In from 2 weeks to a month after the removal of cod liver oil from the diet the fur became normal and the legs lost some of the stiffness. The small crippled animal never recovered. These findings agree with the recent report by Wadsen, McCay, and Maynard ('33), who studied the possible relationship between cod liver oil and muscular degeneration of *Herbivora* fed synthetic diets. Their rabbits re-

ceiving 2 to 3 per cent of cod liver oil in a synthetic ration usually died in 30 to 40 days. Our animals did not die but our diet furnished some naturally occurring foods.

#### EXPERIMENTS WITH RATS

Young rats first depleted of their store of vitamin A were fed monkey 'cookie,' tomato serum, or monkey liver to test the vitamin A content of these substances. Chart D shows typical animals used for the tests. As rats  $M_5$  and  $M_1$  show,

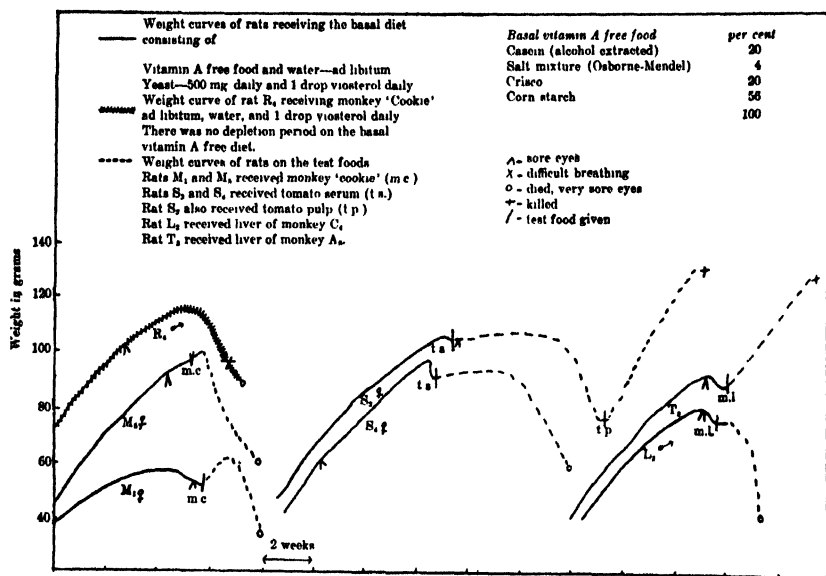


Chart D Weight curves of rats used for testing vitamin A content of monkey 'cookie,' monkey livers, tomato serum, and tomato pulp.

a temporary gain in weight occurred when monkey 'cookie' replaced the synthetic diet but the sore eyes never improved and the rats died in 2 to 3 weeks. They were in a very emaciated condition. When tomato serum was given to the depleted animals, they gained very little weight but they did usually survive for a period of 5 to 6 weeks. The condition of the eyes never improved. Neither with the monkey 'cookie' or tomato serum alone or together as a source a vitamin A did the vitamin-depleted rats ever live through the 8 weeks' test

period usually specified for determining a unit of vitamin A. It must be concluded, therefore, that the monkey diet was very low in vitamin A. The number of units of vitamin A ingested daily by the monkeys, guinea pigs, rabbits, and rats on the vitamin A-low diet was extremely small.

Young and mature rats were also fed the monkey diet with tomato pulp for added vitamin A to show the adequacy of the diet in all respects except vitamin A. Young rats thus

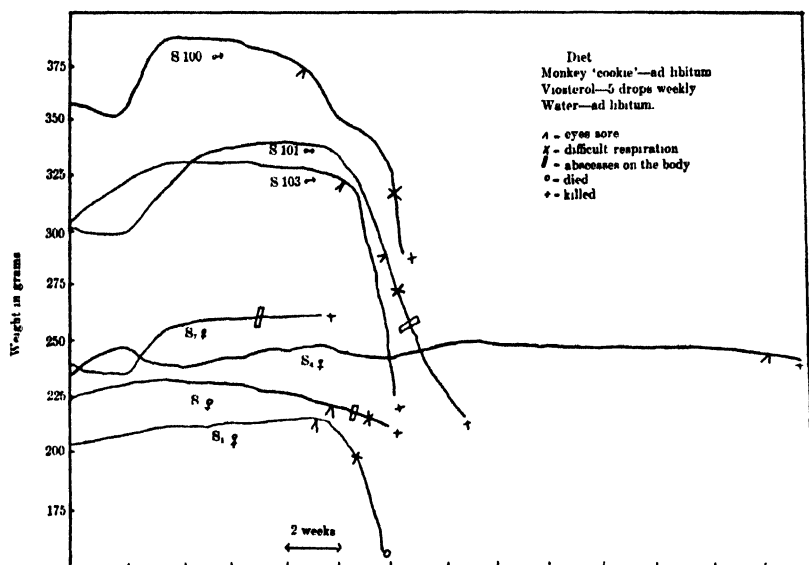


Chart E Weight curves of adult rats receiving a diet low in vitamin A.

fed gained from 2 to 3 gm. daily, which is an average normal rate of growth for a young rat. Mature females became pregnant on this diet and were successful in raising some of their young. The young, however, had very little reserve of vitamin A as is shown in the record of rat  $R_4$ , transferred immediately after weaning to the monkey diet. This animal had sore eyes in 3 weeks and was dead after 2 months on the monkey diet. The average rate of growth and the ability to reproduce on the deficient diet with added vitamin A indicates that it was adequate in all respects except vitamin A.

Adult rats were fed the same vitamin A-low diet given to the monkeys to determine how long full-grown rats could live on the monkey diet before xerophthalmia occurred. These rats usually maintained a fairly constant weight, as chart E shows, for at least 2 months and one animal was killed after 7 months with very little weight loss. Xerophthalmia occurred in 2 to 3 months in all but one animal used, and after 7 months her eyes were beginning to show some soreness. These adult animals studied usually showed difficulty in breathing about the time the eyes became sore and some of the animals had large abscesses on their bodies. One rat, S<sub>7</sub>, was killed before the eyes became sore, for in less than 2 months her shoulder was swollen and filled with pus. Histological study was made only of the eyes of these full-grown animals. They showed advanced xerosis of the epithelium of the cornea and of the bulbar and palpebral conjunctivae. There was usually keratomalacia or incipient keratomalacia of one or both eyes with the same typical specific changes of the epithelial tissue characteristic of young rats on a vitamin A-free diet.

#### SUMMARY AND CONCLUSIONS

Typical xerophthalmia with keratomalacia developed in both of the eyes of one of the twenty-seven monkeys fed the diet low in vitamin A. Histological study of the tissues of this monkey showed keratinization of the epithelial structures of the eyes, the ears, the maxillary sinuses, the turbinates of the nasal septum, the salivary glands, and the kidneys. Histological study showed a normal gastro-intestinal tract. The compact bones immediately underlying the lining membrane of the bulla of the ear showed evidence of decalcification.

Rabbits, guinea pigs, and both young and adult albino rats fed the diet low in vitamin A developed xerophthalmia in most of the cases studied. Histological examination of the ears of the rabbits showed the same tissue metaplasia and bone decalcification that was observed in the ears of the depleted monkey.

The data here reported show that the monkey and the guinea pig may react to vitamin A depletion in the same specific manner as observed in the case of other animals. The storage of reserve vitamin A in the tissues and the resistance to infection during the depletion period may have been the causes for some of the apparently variable findings.

I wish to express my thanks and indebtedness to Dr. Harvey D. Lamb, of the Department of Ophthalmology, to Dr. Howard A. McCordock, of the Department of Pathology, and to Dr. Wm. F. Wenner, of the Department of Oto-Laryngology, who were kind enough to examine the microscopic tissue preparations; to Dr. Howard A. McCordock, who was kind enough to autopsy many of the monkeys, and to Doctor McCordock and to Dr. Donald M. Hetler, of the Department of Bacteriology, for the microphotography. I wish also to thank Dr. William M. James, of the Department of Ophthalmology, who devoted considerable of his time to frequent examination of the eyes of the animals under observation in this study.

#### LITERATURE CITED

- BEACH, J. R. 1923 'Vitamin A' deficiency in poultry. *Science*, vol. 58, p. 542.
- BOOCK, E., AND J. TREVAN 1922 The food value of mangolds and the effects of deficiency of vitamin A on guinea pigs. *Biochem J.*, vol. 16, p. 780.
- DANIELS, A. L., M. E. ARMSTRONG AND M. K. HUTTON 1923 Nasal sinusitis produced by diets deficient in fat soluble A vitamin. *J. Am. Med. Assoc.*, vol. 81, p. 828.
- HART, G. H., S. W. MEAD AND H. R. GUILBERT 1933 Vitamin A deficiency in cattle under natural conditions. *Proc. Soc. Exper. Biol.*, vol. 30, p. 1230.
- MADSEN, L. L., C. M. MCCAY AND L. A. MAYNARD 1933 Possible relationship between cod liver oil and muscular degeneration of Herbivora fed synthetic diets. *Proc. Soc. Exper. Biol.*, vol. 30, p. 1434.
- MCCARRISON, R. 1920 Effect of deficient dietaries on monkeys. *Brit. Med. J.*, vol. 1, p. 249.
- NELSON, V. E., AND A. R. LAMB 1920 Effects of vitamin deficiency on various species of animals; production of xerophthalmia in rabbit. *Am. J. Physiol.*, vol. 51, p. 530.
- SAIKI, S. 1929 Liver function of animals fed on vitamin deficient diets. *Acta Scholae Med. Univ. Imp. Kioto*, vol. 12, p. 157.



- STEENBOCK, H., E. M. NELSON AND E. B. HART 1921 Fat-soluble vitamins; incidence of ophthalmic reaction in dogs fed a fat-soluble vitamin deficient diet. *Am. J. Physiol.*, vol. 58, p. 14.
- STEENBOCK, H., AND I. M. SCHRADER 1931 The distribution of vitamin A in tomato and the stability of added vitamin D. *J. Nutrition*, vol. 4, p. 267.
- STIMSON, A. M., AND O. F. HEDLEY 1933 The effects of vitamin A deficiency diet in dogs. *Public Health Report*, vol. 48, p. 445.
- TILDEN, E. B., AND E. G. MILLER, JR. 1930 The response of the monkey (*Macacus rhesus*) to withdrawal of vitamin A from the diet. *J. Nutrition*, vol. 3, p. 121.
- TURNER, R. G., AND E. R. LOEW 1932 Effect of vitamin withdrawal on the monkey (*Macacus rhesus*). *J. Nutrition*, vol. 5, p. 29.
- WILSON, J. R., AND P. O. DUBOIS 1923 Keratomalacia in infant, with post-mortem examination. *Am. J. Dis. Child.*, vol. 26, p. 431.
- WOLBACH, L. B., AND P. R. HOWE 1925 Tissue changes following deprivation of fat-soluble A vitamin. *J. Exper. Med.*, vol. 42, p. 753.
- 1928 Vitamin A deficiency in the guinea pig. *Arch. Path.*, vol. 5, p. 239.
- WOLFE, J. M., AND H. P. SALTER, JR. 1931 Vitamin A deficiency in the albino mouse. *J. Nutrition*, vol. 4, p. 185.

## PLATES

Descriptions of photomicrographs showing histological changes in tissues of animals on a vitamin A-deficient diet. A few sections are shown from animals on a normal diet.

## PLATE 1

### EXPLANATION OF FIGURES

2 Section through cornea in incipient healing after sloughing of the central part of the cornea from keratomalacia, showing prolapsed iris, ingrowth of anterior epithelium of the cornea around the perforating wound edges and desquamation of superficial keratinized layers of the corneal epithelium to the right of the open wound. Monkey 125.

3 Section through one side of the cornea, showing prolapsed pupillary part of the iris to the left, organizing cellular exudative material to the right, thickening of the anterior epithelium of the cornea, thin layers of edematous fluid between the individual deeply lying epithelial cells, keratinization and desquamation of the superficial epithelial layers of the anterior corneal epithelium. Monkey 125.

4 Section through bulbar conjunctiva, showing metaplasia of the covering epithelium to stratified squamous cell form, with keratinization and desquamation of the superficial flattened cell layers, cystic degeneration of the epithelial cells of the basal layers and a downgrowth of the covering epithelium; moderate infiltration of the subepithelial connective tissue with round and pus cells. Monkey 125.

5 Section through palpebral conjunctiva, showing metaplasia of the covering epithelium to a stratified squamous cell form with keratinization and desquamation of the superficial layers of flattened cells; moderate infiltration of the subepithelial connective tissue with round cells and a few pus cells. Monkey 125.

6 Stratified squamous epithelium replacing the normal columnar lining of large duct in the submaxillary gland of monkey 125.



## PLATE 2

### EXPLANATION OF FIGURES

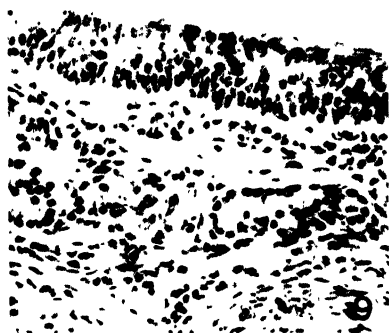
7 Mucous membrane from maxillary sinus in the region of the ostium. Section shows hyperplasia and metaplasia of the epithelium, great degree of keratinization and round cell and plasma cell infiltration in basement membrane and glandular region. Monkey 125.

8 Mucous membrane from the lateral wall of maxillary sinus. Section shows hyperplasia of epithelium, absence of cilia, infiltration of round cells and plasma cells in basement membrane and pus in the lumen. Monkey 125.

9 Normal mucous membrane from maxillary sinus of normal monkey. Magnification the same as in figure 10.

10 Section of mucosa from posterior third of nasal septum shows keratin formation, metaplasia of epithelium and infiltration of neutrophiles, lymphocytes and plasma cells in the basement membrane with emigration of neutrophiles into the lumen passing between the epithelial cells. Monkey 125.

11 Section of mucous membrane from anterior region of middle nasal concha, showing high degree of keratinization, hyperplasia and metaplasia of epithelium and cellular infiltration. Monkey 125.



## PLATE 3

### EXPLANATION OF FIGURES

12 Low-power photograph of the kidney pelvis, showing squamous cell metaplasia of the epithelium with abundant keratin production. Monkey 125.

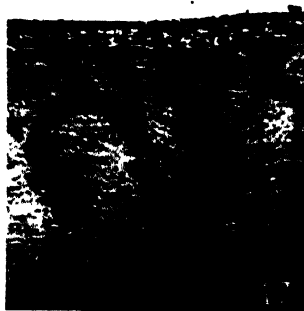
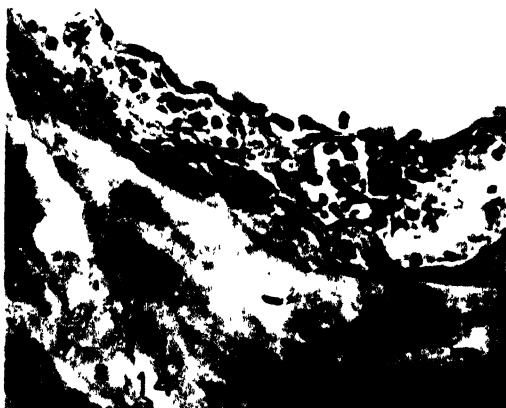
13 High-power photograph of the kidney pelvis as shown in figure 12.

14 Section from mastoid region of the temporal bone of monkey 125. The blood vessels between the epithelial cells and the periosteum are markedly enlarged. The compact bone shows evidence of decalcification.

15 Section from mastoid region of temporal bone of monkey, showing normal lining membrane and normal compact bone. Magnification same as in figure 14.

16 Section of bulba of the temporal bone of rabbit, showing enlargement and engorgement of vessels in lining membrane and decalcification of bone.

17 Section of bulba of the temporal bone of normal rabbit, showing normal lining membrane and compact bone. Magnification the same as in figure 16.





## PLATE 4

### EXPLANATION OF FIGURES

18 Section through one side of rabbit's cornea in the condition of complete sloughing from keratomalacia, showing prolapse of the edematous iris.

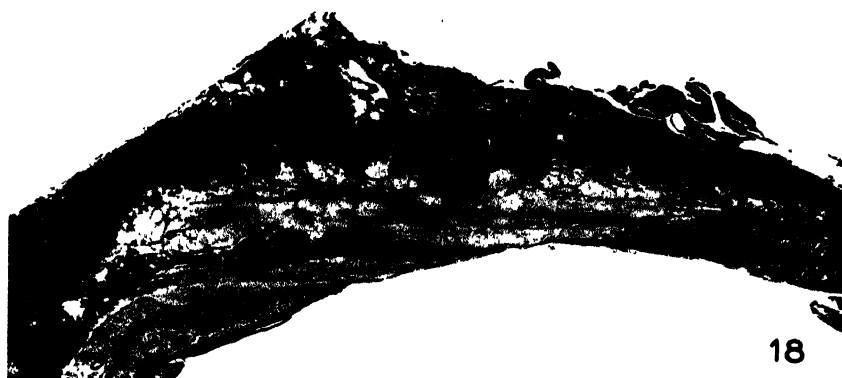
19 Section through anterior corneal lamellae of rabbit's cornea in incipient keratomalacia, showing necrosis and infiltration with pus cells of the superficial layers and a false membrane of degenerated pus cells on the surface.

20 Section through bulbar conjunctiva of a rabbit's cornea, showing metaplasia of the covering epithelium to a stratified squamous cell form with keratinization of the superficial layers of flattened cells and irregular mild cystic degeneration of deeply lying epithelial cells.

21 Mucous membrane of nasal septum of rabbit, showing metaplasia and keratinization of epithelium.

22 Section through superficial layers of cornea of guinea pig, showing an intensive degree of keratinization and desquamation of the anterior epithelium.

23 Section through the inner half of the eyelid of a guinea pig, showing the metaplasia from stratified columnar to stratified squamous cell epithelium with drying and desquamation of the superficial layers of flattened cells.





# VITAMIN D IN THE BLOOD AND MILK OF COWS FED IRRADIATED YEAST

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The nutritive properties of milk have been very extensively studied. The effect of the composition of the diet on the vitamin content of milk has been the subject of numerous investigations. The dairy cow naturally presents the most advantageous subject for this work. The statements in this paper about milk, unless otherwise indicated, refer to cows' milk. The concentration of the vitamins A and D, and probably C and G, in the cow's milk can be influenced by the ration fed. It has not thus far been possible to affect the vitamin B content of cows' milk (Bechdel and Honeywell, '27; Hunt et al., '31; Kieferle, '31; and MacLeod et al., '32). Bechdel ('28) has shown that micro-organisms elaborate vitamin B in large quantities in the rumen. Consequently, supplements of vitamin B in the food may not affect the level of this vitamin in cows' milk.

The vitamin A concentration in the milk is directly influenced by the amount of this vitamin which has been or is being fed the cow, as shown by Kennedy and Dutcher ('23), Moore ('32), Fraps and Treichler ('32) and Hilton, Hauge and Wilbur ('33).

Recent work (Wachtel, '29; Steenbock et al., '30; Krause et al., '32; Hess et al., '31) has shown that the vitamin D content of milk is almost directly proportional to the vitamin D content of the food. It has been shown (Hess et al., '32) that only about 3 per cent of the vitamin D fed is secreted in

the milk, and that 27 per cent is accounted for in the feces. We were interested in determining the concentration of the vitamin D in the blood as related to the concentration of this factor in the food and milk.

#### EXPERIMENTAL

The material used in this study was obtained from the dairy farm of the Walker-Gordon Laboratory Company. A Holstein cow was selected from the herd which was being fed 10 ounces of 30-D yeast daily in three equal portions at 8-hour intervals. She was producing 30 pounds of milk a day. At the beginning of the experimental period, 7 A.M., June 30th, she was fed 10 ounces of 30-D yeast. This was three times the ordinary single dose. No further supplement of irradiated yeast was fed to her until after the experimental period was over. Blood samples were collected at intervals for a period of 50 hours after feeding the yeast. Milk samples were collected at 8-hour intervals for the same period. The blood was immediately centrifuged, and the blood plasma was preserved for bio-assay. Previous work (Hess et al., '32) had shown that practically all of the vitamin D in cows' blood is contained in the plasma. The per cent butter fat in the milk samples was determined, the butter fat separated and stored for bio-assay.

The bio-assays were carried out according to the method outlined by the Wisconsin Alumni Research Foundation, and the results expressed in terms of Steenbock units.

The data presented in table 1 indicate a higher rate of destruction of the vitamin D when the concentration of this factor is high in the blood. The table also illustrates very clearly the relationship between the vitamin D concentration in the blood and in the milk.

This first experiment did not show definitely how quickly after feeding the irradiated yeast the concentration of vitamin D in the blood would be at its maximum. We therefore conducted another experiment on a cow producing 30 pounds of milk daily, taken from the regular herd producing vitamin D

milk. The cow was fed her regular supplement of yeast, which in this case amounted to 1.43 ounces (one-third of the daily amount) of 70-D yeast.<sup>1</sup> Blood samples were taken at

TABLE 1

*Data illustrating the relationship between the vitamin D in the food, the blood, and the milk*

DATE	TIME	HOURS ELAPSED SINCE LAST FEEDING IRRADIATED YEAST	NUMBER OF VITAMIN D UNITS PER GRAM OF BLOOD PLASMA	NUMBER OF VITAMIN D UNITS PER QUART OF MILK PRODUCED
June 30	7 A.M.	Fed 10 ounces of 30-D yeast		
June 30	9 A.M.	2	4	
June 30	1 P.M.	6	2.5	
June 30	9 P.M.	14	1.3	
June 30	10:30 P.M.	15½		266
July 1	3 A.M.	20	1	
July 1	9 A.M.	26	0.66	
July 1	7 A.M.	24		200
July 2	9 A.M.	50	0.5	
July 2	7 A.M.	48		120

A cow which had been receiving 10 ounces of yeast daily in three portions, was given a single feeding of 10 ounces of 30-D yeast at 7 A.M. on June 30th. No further feeding of yeast was given to this cow, and her blood and milk were tested at intervals to determine the amount of vitamin D present.

TABLE 2

*Influence of feeding vitamin D on the vitamin D content of blood*

BLOOD SAMPLE NO.	NUMBER OF HOURS AFTER FEEDING VITAMIN D THAT BLOOD WAS TAKEN FROM COW	UNITS OF VITAMIN D PER GRAM OF BLOOD PLASMA
1	0	1.2
2	1	0.8
3	2	3
4	3	3
5	4	3

the time of feeding the yeast, and at hourly intervals thereafter for 4 hours. The blood was centrifuged, and the plasma was bio-assayed. The results of the assays are given in table 2. These data indicate that the absorption of the vita-

<sup>1</sup> The yeast used in these experiments was that prepared for the commercial production of vitamin D milk. In the interval between the first and second experiments the potency of this yeast was increased from 30-D to 70-D.

min D begins between 1 and 2 hours after it is ingested. The level of the vitamin D in the blood continues to decline for the first hour after feeding the irradiated yeast. From the second to the fourth hours after the feeding of the yeast the level of vitamin D in the blood remains remarkably constant.

The results shown in both tables indicate that a surprisingly high percentage of the vitamin D fed the cow is absorbed into the blood stream. Calculations based on, first, the amount of vitamin D fed; second, the increase in concentration of vitamin D in the plasma following feeding; and, third, the estimated volume of blood plasma, indicate that practically 100 per cent of the vitamin D fed appears in the blood. The amount of vitamin D which disappears from the blood cannot be accounted for either in the milk or in the feces. It is not believed that the fraction unaccounted for is stored in the body, inasmuch as the livers of cows which for months have been approximately 70,000 units of vitamin D in excess of the amount secreted in the milk or excreted in the feces, have only one unit of vitamin D per gram of fresh liver tissue at the end of this time.

#### DISCUSSION

Numerous studies have shown that the transfer of vitamins from the food into the milk is small in relation to the total number of units fed. Sure ('28) has shown that lactating rats require from four to five times the amount of vitamin B necessary for their own bodily needs in order to produce milk containing sufficient of this factor for the nursing young. His work indicates that probably less than 30 per cent of the vitamin B ingested is secreted in the milk. Fraps and Treichler ('32) obtained milks containing as low as 75 to 100, and as high as 2000 units of vitamin A per quart, depending on the vitamin A content of the feeds.

The work of Hess, Light, Frey and Gross ('32) indicates that about 2 to 3 per cent of the vitamin D is secreted into the milk. This figure varies slightly according to variations in the production of the cows. The vitamin D potency of the

butter fat is, roughly, inversely proportional to the per cent butter fat present in the milk, and nearly directly proportional to the amount of vitamin D fed.

The data presented in this paper indicate that practically all the vitamin D ingested is absorbed into the blood stream, although, as shown previously (Hess et al., '32), the major portion is destroyed in the body. Part is re-excreted into the intestinal tract, and is found in the feces. The data also indicate that there is a much more rapid decrease of the vitamin D in the body than had heretofore been suspected.

The secretion of vitamins A and D into milk is similar in one respect, namely, that in the neighborhood of 2 to 3 per cent of the ingested vitamins are secreted into the milk. They differ, however, in that vitamin A appears to be much more stable in the body than vitamin D, and much of the excess vitamin A fed is stored in the liver, where it may act as a reserve supply when the foods contributing this factor are lacking in the ration (Emmett et al., '32; Moore, '31).

Our results would seem to indicate that more work should be undertaken to show how long an excess above that of a normal therapeutic dose of vitamin D will remain in the body. Accurate data would be valuable as an index of the importance of regular supplies of this vitamin. This method of studying the course of the metabolism of vitamin D may also afford an explanation of the differences in therapeutic value which various sources of vitamin D have on different species. Since vitamin D disappears so rapidly from the blood stream, due probably to the fact that it is destroyed, it is not unlikely that substances present in the blood stream or in the vitamin D carrier may influence its rate of disappearance and consequently its effectiveness.



## CONCLUSIONS

1. The vitamin D present in irradiated yeast is practically completely absorbed.

2. Evidence points to a rapid disappearance of vitamin D from the blood stream at a maximum rate of approximately 10 per cent per hour followed by a decline in the rate of destruction as the concentration decreases.

3. The concentration of vitamin D in the blood plasma governs the concentration of this factor in the milk.

## LITERATURE CITED

- BECHDEL, S. I., AND H. E. HONEYWELL 1927 The relation between the vitamin B content of the feed eaten and of the milk produced. *J. Agri. Research*, vol. 35, p. 283.
- BECHDEL, S. I., H. E. HONEYWELL, R. A. DUTCHER AND M. H. KNUTSEN 1928 Synthesis of vitamin B in the rumen of the cow. *J. Biol. Chem.*, vol. 80, p. 231.
- EMMETT, A. D., O. D. BIRD, E. NIELSEN AND H. J. CANNON 1932 A study of halibut-liver oil. I. With respect to its vitamin potency physical constants, and tolerance. *Ind. and Eng. Chem.*, vol. 24, p. 1073.
- FRAPS, G. S., AND R. TREICHLER 1932 Quantitative variations in vitamin A content of butter fat. *Ind. and Eng. Chem.*, vol. 24, p. 1079.
- HESS, A. F., J. M. LEWIS, F. L. MACLEOD AND B. H. THOMAS 1931 Anti-rachitic potency of the milk of cows fed irradiated yeast or ergosterol. A clinical test. *J. Am. Med. Assn.*, vol. 97, p. 370.
- HESS, A. F., R. F. LIGHT, C. N. FREY AND J. GROSS 1932 A study of the milk, blood, and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol. *J. Biol. Chem.*, vol. 97, p. 369.
- HILTON, J. H., S. M. HAUGE AND J. W. WILBUR 1933 Maintaining the vitamin A value of butter through winter feeding conditions. *J. Dairy Science*, vol. 16, p. 355.
- HUNT, C. H., AND W. E. KRAUSS 1931 The influence of the ration of the cow upon the vitamin B and vitamin G content of the milk. *J. Biol. Chem.*, vol. 92, p. 631.
- KENNEDY, C., AND R. A. DUTCHER 1922 Influence of the diet of the cow upon the quantity of vitamins A and B in the milk. *J. Biol. Chem.*, vol. 50, p. 339.
- KIEFERLE, F. 1931 Influence of feeding with irradiated dry yeast upon milk and its nutritive value. Conference papers International Dairy Congress, Copenhagen, sect. 1, p. 166.
- KRAUSS, W. E., R. M. BETHKE AND C. F. MONROE 1932 The effect of feeding irradiated ergosterol to cows on the vitamin D content of milk. *J. Nutrition*, vol. 5, p. 467.

- MACLEOD, R. I., J. B. BRODIE AND E. R. MACLOON 1932 The vitamin A, B and G contents of milk throughout the year. *J. Dairy Science*, vol. 15, p. 14.
- MOORE, THOMAS 1931 Vitamin A and carotene. VII. The distribution of vitamin A and carotene in the body of the rat. *Biochem. J.*, vol. 25, p. 275.
- 1932 Vitamin A and carotene. IX. Notes on the conversion of carotene to vitamin A in the cow. *Biochem. J.*, vol. 26, p. 1.
- STEENBOCK, H., E. B. HART, F. HANNING AND G. C. HUMPHREY 1930 Fat-soluble vitamins. XXX. The anti-rachitic value of cow's milk as modified by the feeding of irradiated yeast. *J. Biol. Chem.*, vol. 88, p. 197.
- SURE, BARNETT 1928 The inefficiency of the lactating mother (*Mus norvegicus albinus*) in secreting vitamin B in the milk, and the relation of this phenomenon to infant mortality. *J. Biol. Chem.*, vol. 76, p. 685.
- WACHTEL, M. 1929 Irradiated yeast as means to increase quantity and vitamin content of mother's milk and of cow's milk. *Münch. med. Woch.*, Bd. 76, S. 1513.



# THE USE OF THE METHOD OF PARTIAL REGRESSION IN THE ANALYSIS OF COMPARATIVE FEEDING TRIAL DATA,<sup>1</sup> PART I

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## 1. OBJECT OF STUDY

From the standpoint of the clarity of interpretation of results, an ideal feeding trial intended to evaluate feeds or rations would be one in which all the animals were alike at the start and consumed equal quantities of feed during the test. This condition is never attained in actual practice, and various procedures are employed to correct the results for the effects which the variations in the experimental animals have on them. Through careful allotment, individual differences in age, sex, breeding, thrift, condition, etc., are equalized as far as possible between experimental lots. This appears at the present time to be the only practicable method of dealing with those factors which do not lend themselves to accurate description in numerical terms. Its efficiency will depend in a large measure on the skill of the operator.

Initial weight and feed consumption, however, are usually treated differently. Differences in the initial weights of the animals are first equalized as well as possible between lots by the allotment procedure and then further corrected for in

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the analysis of the trial by subtracting them from the final weights to obtain the gains in live weight. These gains, rather than the actual weights themselves, are generally employed in interpreting the results of the trial. Then corrections for varying feed consumption for purposes of analysis and interpretation of the test have been attempted by calculating a simple gain-feed ratio.

This method of equalizing by allotment the final weights for differences in initial weights is restricted in use to the original assortment of animals into feeding groups, which in many cases may be at the start of a preliminary feeding period. Nor is the calculation of the gain always the most efficient procedure, since, if the correlation between initial and final weights is low or negative, the standard deviation of the gains will be larger than that of the final weight. ( $s_D^2 = s_I^2 + s_F^2 - 2 r s_I s_F$ .)

The use of the gain-feed ratio as the basis for statistical analysis of feeding trial data appears to demand two assumptions: a) that gain is independent of initial weight and, b) that gain is directly proportional to feed intake.

That gain is not independent of initial weight is shown by correlation studies presented later in this paper. Furthermore, previous work by one of us (Crampton, '33) has shown that gains are not directly proportional to feed intake, presumably since a certain amount of feed must be supplied a pig for maintenance alone. Consequently, even if the correlation between gain and feed eaten be perfect so that the gain is uniquely defined as a linear function of the feed, that function will not be of the form  $g = bf$  but rather  $g = a + bf$ . The gain per 100 pounds feed then becomes:

$$\frac{100 (a + bf)}{f}$$

which is not a constant but a variable quantity tending to the limit  $b$  as  $f$  is increased indefinitely. As in practice  $f$  cannot be increased indefinitely but assumes various finite values, ratios obtained will be subject to systematic errors.

When this calculation is made for each pig of the trial, a statistical analysis of the array of ratios obtained will include, in addition to the experimental error, a systematic error which may be of sufficient magnitude seriously to distort the result, and to vitiate the estimate of experimental error and hence any test of significance.

This paper reports a study of the relationships which exist between the initial weights, feed consumption and live weight gains of swine. The use, in the interpretation of feeding data, of the method of partial regression as a means of correcting live weight gains for the effects of differences in the initial weights of the experimental animals and of varying feed consumption during the feeding period is also discussed.

## 2. OBSERVATIONAL DATA

The material studied consists of results of a fairly extensive series of feeding trials at Macdonald College in which the rations and treatment of the pigs were comparable. Individual, hand feeding was employed in all cases and individual feed records are available for each animal. Pigs were weaned at approximately 60 days of age and went onto test immediately.

The data thus available were divided into four series, according to the class of pig and its treatment:

Series A. Pigs fed for 30 days, beginning at weaning or at approximately 60 days of age.

Series B. Pigs fed for 60 days, beginning 30 days after weaning or at about 90 days of age.

Series C. Pigs fed for 30 days, beginning 90 days after weaning or at about 150 days of age.

Series D. A combined growing and finishing period. It covered 75 days of feeding, and included one change in ration at the end of 30 days at which time the proportion of protein supplement was reduced from 20 per cent to 10 per cent of the ration.

The rations used were as follows:

	<i>Basic feeds</i> <sup>1</sup> Per cent	<i>Protein-mineral supplement</i> <sup>2</sup> Per cent
Series A, 30 days	70	30
Series B, 60 days	80	20
Series C, 30 days	90	10
Series D { 30 days	80	20
45 days	90	10

<sup>1</sup> Basic feeds consisted of corn, barley, hominy, wheat or mixtures of them.

<sup>2</sup> Standard Macdonald protein-mineral supplement—40 tankage, 20 fish meal, 20 linseed oilmeal, 10 bone meal, 7.6 limestone, 2 salt, 0.4 ferric oxide.

Table 1 gives a summary of the analysis of the variance and covariance of the initial weights, feed intake and gains of the animals in the various classes.

It should be mentioned that while the pigs in series A were originally allotted with due consideration of initial weight, no such sorting was made in any of the other series, since these subsequent periods were but parts of longer trials, the original allotment for which took place at the beginning of the respective weanling periods. This, in addition to the fact that differences in weights between pigs of the same ages would naturally tend to be greater in older than in younger pigs, accounts for the differences between the four series in variation of initial weights.

### 3. STATISTICAL ANALYSIS AND RESULTS

As will be seen from table 1, there are, excepting initial weight and gain in series D, highly significant correlations between initial weights, feed eaten, and gains in weight made by the pigs.

These relationships may, moreover, be utilized to correct the observed gains for differences in initial weight and feed consumption, and thus markedly to reduce the variability of the results, without introducing those errors inherent in the gain-feed ratio, to which attention has been directed.

Before proceeding to a consideration of the practical application of the method, however, it may be of interest to examine somewhat more closely the nature of the relation-

ship between initial weight, feed consumption, and gains, in the four series. The crude values of the coefficients of correlation between gain and feed intake and between gain and

TABLE 1

*Standard deviations of and correlations between initial weight, feed eaten and gain in live weight of swine of different age groups*

VARIABLES	MEAN	VARIANCE OR CO- VARIANCE	S.D.	C.V.	r	r NECESSARY FOR P = .01
Series A (N = 167)						
Initial weight, pounds ( $x_1$ )	28.6	42.59	6.53	22.83	...	...
Feed eaten, pounds ( $x_2$ )	106.5	408.66	20.22	18.99	...	...
Gain in weight, pounds ( $y_1$ )	38.3	67.08	8.19	21.38	...	...
Initial weight $\times$ gain ( $x_1 y_1$ )	....	23.49	....	....	0.44	0.20
Initial weight $\times$ feed ( $x_1 x_2$ )	....	80.39	....	....	0.61	0.20
Feed eaten $\times$ gain ( $x_2 y_1$ )	....	147.90	....	....	0.89	0.20
Series B (N = 69)						
Initial weight, pounds ( $x_1$ )	64.1	155.51	12.47	19.45	...	...
Feed eaten, pounds ( $x_2$ )	391.7	2411.22	49.10	12.54	...	...
Gain in weight, pounds ( $y_1$ )	94.6	227.47	15.08	15.94	...	...
Initial weight $\times$ gain ( $x_1 y_1$ )	....	71.24	....	....	0.38	0.30
Initial weight $\times$ feed ( $x_1 x_2$ )	....	402.30	....	....	0.66	0.30
Feed eaten $\times$ gain ( $x_2 y_1$ )	....	498.06	....	....	0.67	0.30
Series C (N = 89)						
Initial weight, pounds ( $x_1$ )	155.5	471.40	27.71	13.96	...	...
Feed eaten, pounds ( $x_2$ )	240.8	802.25	28.32	11.76	...	...
Gain in weight, pounds ( $y_1$ )	51.3	86.65	9.31	18.14	...	...
Initial weight $\times$ gain ( $x_1 y_1$ )	....	100.19	....	....	0.50	0.27
Initial weight $\times$ feed ( $x_1 x_2$ )	....	344.23	....	....	0.56	0.27
Feed eaten $\times$ gain ( $x_2 y_1$ )	....	145.80	....	....	0.55	0.27
Series D (N = 48)						
Initial weight, pounds ( $x_1$ )	67.6	129.30	11.37	16.81	...	...
Feed eaten, pounds ( $x_2$ )	514.8	4564.75	67.56	13.12	...	...
Gain in weight, pounds ( $y_1$ )	125.5	247.53	15.73	12.53	...	...
Initial weight $\times$ gain ( $x_1 y_1$ )	....	36.55	....	....	0.20	0.35
Initial weight $\times$ feed ( $x_1 x_2$ )	....	575.41	....	....	0.75	0.35
Feed eaten $\times$ gain ( $x_2 y_1$ )	....	624.79	....	....	0.59	0.35

initial weight given in table 1 suffer from the defect that initial weight and feed consumption are themselves correlated. In order to obtain an estimate of the independent effect



of each, the method of partial correlation must be employed. It will be advantageous to calculate the partial regression coefficients rather than the partial correlation coefficients, for the former are not only of more interest in themselves, but may be used directly to reduce the variability of the gains.

We may proceed to the calculation of the partial regression coefficients for each series by solution of the following simultaneous equations<sup>3</sup>:

$$\begin{aligned} b_1 S(x_1^2) + b_2 S(x_1 x_2) &= S(x_1 y) \\ b_1 S(x_1 x_2) + b_2 S(x_2^2) &= S(x_2 y) \end{aligned}$$

where  $b_1$  and  $b_2$  are the partial regression coefficients of gain on initial weight and feed consumption, respectively; and  $x_1$  = initial weight,  $x_2$  = feed eaten,  $y$  = gain.  $S$  stands for the summation of all sets of observations corrected to their respective means, i.e.,  $S(x_1^2) = S(x_1 - \bar{x}_1)^2$ , etc.

Since to test the significance of the regression coefficients we shall need to know the values of  $c_{11}$  and  $c_{22}$ ,<sup>4</sup> it will be somewhat simpler first to obtain the  $c$  values by writing the equations:

$$\begin{aligned} b_1 S(x_1^2) + b_2 S(x_1 x_2) &= 1, 0 \\ b_1 S(x_1 x_2) + b_2 S(x_2^2) &= 0, 1 \end{aligned}$$

and the solutions of the two sets of equations as—

$$\begin{aligned} b_1 &= c_{11}, c_{12} \\ b_2 &= c_{21}, c_{22} \end{aligned}$$

The regression coefficients may then be found by substituting in the formulas:

$$\begin{aligned} b_1 &= c_{11} S(x_1 y) + c_{12} S(x_2 y) \\ b_2 &= c_{21} S(x_1 y) + c_{22} S(x_2 y) \end{aligned}$$

The results thus obtained from series A, B, C and D are given in table 2.

From table 2 some interesting inferences may be drawn. First, it should be noted that all of the partial regression coefficients of gain on feed consumption are highly significant,

<sup>3</sup> See Fisher, R. A., Statistical methods for research workers, p. 132, 3rd ed.

<sup>4</sup> See Fisher, R. A., Statistical methods for research workers, section 29.

$t$  for  $P = .01$  with  $n$  greater than 30, being given by Fisher as 2.58. The decreasing rate at which gains are made per unit of feed eaten with advancing age of pig is also clearly shown.

The effects of initial weight on gains are perhaps surprising at first glance. It would seem that for weanling pigs and for trials involving young pigs fed to or nearly to market weight, the calculation of gains does not efficiently correct the data for the effects of differences between pigs in initial weight. With older pigs the effects of differences in initial weight on gains are of less importance. It appears that with young growing pigs the larger the pig at the start of the feeding the smaller the gain.

TABLE 2

*Partial regression coefficients, and their standard errors, of gains on initial weight ( $b_1$ ) and feed eaten ( $b_2$ )*

SERIES	N	INITIAL WEIGHT (b)				FEED EATEN (b)			
		$b_1$	Standard error ( $s \sqrt{c_{11}}$ )	$t = \frac{b_1}{s \sqrt{c_{11}}}$	P	$b_2$	Standard error ( $s \sqrt{c_{22}}$ )	$t = \frac{b_2}{s \sqrt{c_{22}}}$	P
A	167	-0.209	0.053	3.94	<0.01	0.403	0.018	22.77	<0.01
B	69	-0.134	0.145	0.92	0.35	0.229	0.032	7.17	<0.01
C	89	0.116	0.272	0.41	0.68	0.132	0.034	3.88	<0.01
D	48	-0.744	0.222	3.35	<0.01	0.230	0.037	6.17	<0.01

The results with series D are particularly interesting, since this group corresponds closely to a common type of hog feeding trial in which young pigs averaging from 60 to 70 pounds are fed to market weight. Here it seems evident that initial weight is of pronounced importance in the gains made by the pigs. Given pigs of the same age and eating equal amounts of feed, each extra  $\frac{3}{4}$  pound of weight at the beginning of the trial results on the average in a penalty of 1 pound of gain at the end of a 75-day feeding period. This may probably be partially accounted for by the fact that extra weight on a young pig of, say, 90 days of age usually implies that the animal is carrying considerable fat and hence not in condition to make most rapid gains for feed eaten. Furthermore, larger

pigs are likely also to be more mature and hence to require more feed for a unit of gain.

By reference to table 1, it appears that in series D initial weight is less closely related to gain than to feed consumption. However, as indicated on page 117, the apparent correlation between any two of these factors is affected by the correlation of both with the third factor. The results given in table 2 indicate that there is, in fact, a significant relation between initial weight and gain. The variability of the initial weights of the pigs in this series is by no means abnormal, being measured by a standard error of about 17 per cent which will compare favorably with that of average feeding trials. (An average of the standard deviations of initial weights reported by Mitchell and Grindley ('13) for some twenty lots of hogs in which the initial weights were comparable to those of this study, i.e., 60 to 70 pounds, is approximately 13.0, or very nearly 20 per cent.)

It is evident that, except possibly during the fattening period, i.e., when the animals have passed the period of rapid growth, both feed and initial weight should be considered for their effects on gains. To omit the factor of initial weight, which is done where the gain-feed ratio is used, is certainly to be questioned. This would be particularly true in the case of a feeding period analyzed by parts.

As an example, take the case in which two or more rations for weanling pigs are to be studied. The experimental difference, such as the addition to one ration of cod liver oil, might apply only during a short period at the start of the trial, as in series A, and then for the remainder of the feeding to market weight the comparative lots be carried on identical rations in order to learn if the cod liver oil fed during the weanling period affected the subsequent progress of the pigs. Obviously, it would be impossible to reallocate the pigs at the end of the cod liver oil feeding sub-period, and hence the initial weights of the pigs for the subsequent period would likely be much more variable and poorly distributed than ideal allotment would call for. Analysis of the gains made during

such subsequent periods, without regard to the effects of varying initial weights, would be less accurate than if this related factor had been taken into account.

#### 4. APPLICATION TO REDUCTION OF VARIANCE

The variance of the individual gains furnishes a direct measure of the precision of any feeding trial, for the significance of experimental differences can only be established by contrasting them with the differences existing among animals treated alike. It has been shown in section 3, however, that, in the present instance at least, differences in initial weight and in feed consumption contribute to this variance; and the regression equation—

$$Y = \bar{y} + b_1 (x_1 - \bar{x}_1) + b_2 (x_2 - \bar{x}_2)$$

may be used to free the observed gains of fluctuations attributable to the above factors. The increase in precision thus effected may be shown by a comparison of variance of the raw gains ( $y$ ) with that of the corrected gains ( $Y$ ). The sum of the squares of ( $y - Y$ ) is easily obtained, since—

$$S(y - Y)^2 = S(y^2) - b_1 S(x_1 y) - b_2 S(x_2 y)$$

and to obtain the variance, therefore, we calculate:

$$s^2 = \frac{1}{n^1 - p - 1} S(y - Y)^2$$

$n$  being the number of observations, and  $p$  the number of coefficients calculated therefrom—in this case two.

Table 3 gives for each series the sum of squares, variance and standard error of the raw gains ( $y$ ) and the gains corrected by the regression coefficients.

TABLE 3  
*Analysis of variance of gains*

SERIES	RAW GAINS				GAINS CORRECTED BY REGRESSION FOR INITIAL WEIGHT AND FEED INTAKE				RELATIVE PRECISION
	D/F	Sum of squares	Variance	Standard error	D/F	Sum of squares	Variance	Standard error	
A	166	11134.6	67.08	8.19	164	2054.2	12.53	3.54	5.4
B	68	15468.2	227.47	15.08	66	8365.8	126.76	11.26	1.8
C	88	7625.2	86.65	9.31	86	6957.3	80.90	8.99	1.1
D	47	11634.1	247.53	15.73	45	6139.8	136.44	11.68	1.8

It is very evident from this table that, except in the case of the fattening pigs (series C), a definite increase in precision is obtained in an analysis of gains by correcting them for the effects of varying initial weights and feed intake. In the case of weanling pigs, for instance, it would be necessary to use fifty-four pigs to obtain as reliable an average gain as could be obtained with ten pigs with gains corrected for these two related factors.

It may be pointed out that this method of analysis is not confined to the items of initial weight, feed intake and gains, but is equally applicable to any of the correlated variables of a trial which can be numerically expressed. For instance, it is the practice in certain cases to remove pigs from test at a predetermined final weight instead of after a fixed length of feeding period, and to use the days on feed as the basis of the analysis. In such cases, the number of days fed would become the dependent variate to be corrected for the effects of initial weight of pigs and feed consumption.

Another possibility which seems to offer certain advantages is to dispense entirely with the gain figure, basing the statistical analysis on the final weights of the pigs corrected by partial regression for effects of variations in initial weight and feed consumption.

#### ANALYSIS OF COMPARATIVE FEEDING TRIALS

In the present communication we have been concerned mainly to demonstrate the relationship existing between gains and initial weight and feed consumption of swine fed on the same ration, and to indicate the manner in which this relationship may be used to reduce the variance of the gains. The method is, however, also applicable to comparative feeding trials, in which the animals do not all receive the same kind of treatment or ration, but are divided into two or more differently treated lots. In such cases the statistical procedure known as the analysis of covariance enables the regression coefficients to be estimated from the variance and covariance within lots, and provides a means not only of reducing the

variance of the results, but also of correcting the observed differences in gains between lots for the disturbing effects of differences in average initial weight and feed consumption.

#### SUMMARY

Statistical analysis by the method of partial regression has been employed to determine the relationship between gains and initial weight and feed consumption of swine of four different age groups. Highly significant relationships between gains and feed consumption are exhibited by all groups, the increase in gain due to each additional unit of feed consumed decreasing with advancing age of pig. In the case of weanling pigs and young pigs fed to market weight, the gain made is also dependent upon the initial weight of the pig.

The gain-feed ratio commonly employed does not efficiently correct the observed final weights for variations in initial weight and feed intake. A satisfactory correction may, however, be made by means of the regression coefficients, markedly reducing the variance of the results without introducing errors inherent in the gain-feed ratio. This procedure is also applicable to comparative feeding trials, in which the animals are divided into two or more differently treated lots.

#### LITERATURE CITED

- CRAMPTON, E. W. 1934 Analysis of the comparative feeding trial by the variance and covariance methods. *J. Nutrition*, vol. 7, pp. 305-320.
- MITCHELL, H. H., AND H. S. GRINDLEY 1913 The element of uncertainty in the interpretation of feeding experiments. *Ill. Agr. Exp. Sta. Bul.* 165.



## IRON AND COPPER RETENTIONS IN YOUNG CHILDREN

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Although iron is an essential constituent of animal protoplasm, the larger proportion existing in the hemoglobin of the blood, there is a paucity of data in the literature regarding the actual amount required by the human organism, especially by children. In fact, only two such studies with children of preschool age were found in the recent literature. Rose and co-workers ('30) have reported a study with a 31-month-old child in which a negative balance was obtained with an ingestion of 0.33 mg. of iron per kilogram, and Leichsenring and Flor ('32) working with four normal children between 3 and 6 years of age obtained retentions of 0.07 and 0.18 mg. per kilogram at ingestion levels of 0.19 and 0.36 mg., respectively. The iron excretion, 0.12 mg. per kilogram, at the lower ingestion level the latter authors interpreted as an index of the maintenance requirement, whereas the retention, 0.2 mg. at the higher level, was the amount needed for growth. The total minimum requirement, therefore, was estimated to be 0.32 mg. per kilogram. To this the authors add 50 per cent as a margin of safety to cover individual differences.

According to recent investigations (Hart, '28; Elden, '28; and Kraus, '29) iron alone cannot effect a formation of hemoglobin; small amounts of copper must be present to stimulate the physiological processes. Therefore, studies of the iron requirements would seem to be incomplete without information regarding the copper requirements.



The amount of copper needed by the human organism apparently is not known. A search of the literature reveals no studies of a quantitative nature, with the exception of determinations of the copper content of various human tissues (Bodansky, '21; Morrison, '30). The concentration of copper in the blood in relation to that of iron might be expected to give some hint as to the relative amounts of these elements which should be ingested. From the data available, the ratio of iron to copper in the blood of different animals was found to vary from 34:1 for the cow (McHargue, '28) to 740:1 for the rat (Lindow, '29). Elvehjem, Steenbock, and Hart ('29) fed a ration to rats in which the ratio of iron to copper was 50:1, a much larger amount of copper than would seem necessary if the concentration of iron and copper in rat blood were taken as a criterion. In children the iron-copper ratio of the blood has been estimated to be 300:1 (Sobel, '33; Gorter, '31). Whether the human organism requires more or less copper in relation to the amount of iron than the rat remains to be determined.

#### EXPERIMENTAL PROCEDURE

In the investigation, iron and copper retentions have been determined in eight normal children, three girls and five boys ranging in age from 3 to 6 years. From one to three studies were made with each child. Two types of diets were fed, varying in the choice of foods, but containing relatively slight differences in the amounts of copper and iron. Although it would have been desirable to study the retentions on diets containing a wider range of iron and copper, the close agreement was necessitated by the fact that the diets were planned to meet the needs of another study. The experimental period consisted of 8 days, the first 3 days for adjusting the children to the diet, and the last 5, the metabolism period. During the study the children, who were under constant supervision, received accurately weighed diets. Urine and feces were collected quantitatively.

The two types of diets (samples of which are given in table 1) differed in that one contained cereal, meat, eggs, vegetables, and fruits; whereas the other although similar, contained a larger amount of cereal foods and no meat. The milk used was either pasteurized or evaporated.

The foods used were purchased in quantities sufficient to last the entire period, the perishable materials being kept in a low-temperature refrigerator. In preparing the diets, the cereals and sugar were weighed dry. At the beginning of each experimental period, potatoes and carrots were pared, cut into inch cubes, and kept in distilled water. In this way a more nearly uniform material was obtained. When these were to be used, they were drained, dried with a towel, weighed and so cooked that there was no excess liquid to be discarded at the end of the process. Each child's food was prepared in separate containers. Prunes and apples for the entire period were cooked in distilled water, sieved to insure a uniform mixture, and weighed after cooking. Bananas were taken from a single stalk. The meat, ground round steak, was weighed raw and cooked in porcelain ramekins in which it was served. The bread for a given child was cut from the same loaf. Sufficient orange juice, as well as canned tomato, was prepared at the beginning of the period. Eggs for the period of study were thoroughly beaten and a portion taken for the day's serving. When evaporated milk was used, one large can sufficed for the 8-day study. The pasteurized milk was obtained fresh each morning and an aliquot from each day's supply was taken for analysis. All foods for the children, as well as the food to be analyzed, were cooked in aluminum or glazed porcelain vessels. The food prepared for analysis was from the same supply as that served the children. Distilled water was used in all cases for cooking and drinking.

Since the milk for analysis was taken from the supply served to the children in a given group, it was necessary to make only one determination for the three balance studies. This was measured into porcelain crucibles, dried in a Freas

TABLE 1  
*Typical diets of children during experimental periods*

NAME	DATE	DIET <sup>1</sup>	PRUNES	OATMEAL	MILK	BREAD, WHOLE WHEAT	ORANGE JUICE	POTATO	MEAT	TOMATO	APPLESAUCE	EGG	CARROTS	BANANAS	BALISTON	SUGAR	BUTTER	COD LIVER OIL
A.C.	2/3	A	60	12.6	487.5	65	120	100	50	110	90	75	70	90	..	27	28	7.2
A.C.	2/11	B	60	12.6	487.5	65	120	100	50	110	90	75	70	90	..	27	28	7.2
A.C.	3/27	C	55	20.0	487.5	100	120	130	..	110	85	60	..	120	20	18	14	7.2
D.B.	4/7	D	40	20.0	487.5	80	120	110	..	110	70	60	..	110	20	15	11	7.2

<sup>1</sup> Diet A. Meat with pasteurized milk.

Diet B. Meat with evaporated milk.

Diet C. High cereal with pasteurized milk.

Diet D. High cereal with evaporated milk.

oven at a temperature of 60°C., and ashed in an electric muffle furnace, 50 cc. being taken for the iron determinations and 250 cc. for copper.

Similarly, analyses were made on composite samples of the day's supply of food prepared in the same way as that fed. This was placed in a large porcelain evaporating dish, thoroughly mixed with acid alcohol, evaporated to dryness on a steam bath, brought to constant weight in a drying oven, ground in a porcelain mortar, and stored in a desiccator until such time as analyses could be made. Aliquot samples of the dried foods were likewise ashed in porcelain crucibles, 5 gm. being taken for the determinations of iron and 20 gm. for copper.

Stools for the 5-day period, marked with 0.2 gm. of carmine, were treated in the same manner as the food, 2 and 5 gm. of the dried material being taken for the copper and iron determinations respectively.

The daily urinary excretions were made up to a given volume with distilled water. Aliquots were pooled and the analyses made of the composite sample, 150 cc. to 270 cc. being used for iron and 1000 cc. to 2000 cc. for copper. The measured amounts were then dried in pyrex beakers on a steam bath, the residues dissolved in concentrated nitric acid and transferred quantitatively to porcelain evaporating dishes, which were heated slowly over an open flame until the nitric acid was driven off and the material completely ashed.

Iron was determined by the method of Elvehjem and Hart ('26) and copper according to the analytical procedures of Elvehjem and Lindow ('29). All precautions suggested by the authors were carefully followed: Distilled water was redistilled from glassware; porcelainware was treated with alcoholic sodium acetate, ignited, and subsequently extracted with 1:1 HCl for at least 4 days. Determinations were made in triplicate with the exception of the copper analysis of urine for which there was an insufficient amount of material. Duplicate determinations were therefore made in all but three cases, in which there was enough material for only one.

Analyses of food, feces, or milk which seemed questionable were repeated.

In order to test the reliability of the technics employed, known amounts of iron and copper were added to given samples of food and feces. The percentage recovery, based on this procedure, was found to be 101.1 per cent in the iron

TABLE 2  
*Tests for accuracy of method*

IRON IN ORIGINAL SAMPLE	IRON ADDED	IRON RECOVERED	IRON RECOVERED
Iron			
<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Per cent</i>
0.7598	0.1000	0.8609	101.1
0.7890	0.1000	0.8922	103.2
0.2442	0.1000	0.3431	98.9
Average:			101.1
COPPER IN ORIGINAL SAMPLE	COPPER ADDED	COPPER RECOVERED	COPPER RECOVERED
Copper			
<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Per cent</i>
0.2564	0.1000	0.3529	96.5
.....	0.1000	0.1000	100.0
0.0870	0.1000	0.1832	96.2
Average:			96.2

analyses and 97.6 per cent for copper (table 2). Blank determinations were made on reagents each time new solutions were prepared, and corrections made on each iron analysis accordingly. Analysis of the reagents used in the copper determinations gave negative results. The distilled water used for drinking and cooking, as well as that used for diluting the urine, contained no copper, and only a faint suggestion of iron, in 800 cc.—too little to test.

## RESULTS AND COMMENTS

*Iron*

Both the iron and copper of the food differed somewhat from the theoretical amounts estimated from analyses reported for iron by Peterson and Elvehjem ('28) and for copper by Lindow, Elvehjem and Peterson ('29). Wide variations in the mineral content of plant foods apparently are to be expected, depending upon conditions under which they are raised (Remington, '30).

The variation in the iron and copper contents of milk was particularly noticeable. The milk analyzed contained from 1.24 to 2.22 mg. of iron per liter, and 0.22 to 0.76 mg. of copper—results which come within the range of values reported by other investigators. For example, Rose found that milk furnished about 0.60 mg. of iron per liter; Leichsenring and Flor reported 1.80 mg., while Peterson and Elvehjem gave the average iron content of milk as 2.24 mg. per liter.

The normal copper content of milk has been reported by Lindow, Elvehjem and Peterson as 0.15 mg. per liter; Supplee and Bellis ('22), using a different method of analysis, found 0.2 to 0.8 mg. per liter; and Quam and Hellwig ('28) obtained results varying from 0.26 to 0.52 mg. The copper contents of the three samples of evaporated milk used in this study were considerably higher (0.33, 0.62, and 0.76 mg. per liter) than the values obtained for the pasteurized milk (0.22, 0.26, and 0.35 mg. per liter). Whether the larger amount was present in the original milk or was due to contamination during the manufacturing process is not clear.<sup>1</sup> Rice and Miscall ('23) and Rice ('26) have shown that copper is dissolved from the vacuum pan during the condensing process in the manufacture of condensed and powdered milk, thus increasing the quantity of copper in these products to a considerable extent. The evaporated milk used in the children's diets, according to a statement by the manufacturers, was condensed under vacuum in copper pans.

<sup>1</sup>Since this manuscript was completed, the report by Stein and Lewis has confirmed our findings regarding the higher copper content of evaporated milk. *J. Nutrition*, 1933, vol. 5, p. 465.

The iron intake of the children studied ranged from 0.57 to 0.75 mg. per kilogram and from 0.60 to 0.75 mg. per 100 Calories of food. These amounts approximate the requirement of 0.61 mg. proposed by Rose et al., but exceed the suggested standard given by Leichsenring and Flor. The ingestions, estimated on the 100 Calorie basis, conform rather closely to the standard of the latter workers, but are somewhat lower than the amounts advocated by Rose; the caloric ingestions of the children in the studies, however, were higher.

From 94 to 97 per cent of the iron excreted was found in the feces. The amount in the urine varied from 0.22 to 0.55 mg. per day. No outstanding relationship between intake and urinary output was noted.

The iron retentions of the children studied varied from 0.12 to 0.27 mg. per kilogram (average 0.18 mg.). At the same ingestion level, A.C. retained 0.24 mg. per kilogram during the first period (table 3) and only 0.15 mg. during the subsequent period: R.H. also retained more during the first metabolism period, whereas B. C. retained practically the same amounts during all three periods. The explanation for the higher retentions during the first metabolism periods may be previous food conditions. These children lived in a children's home where food conditions were good, but perhaps not optimal. Undernourished children are known to retain larger amounts of essential elements than those who are well stocked (Wang, '29; Stearns, '21; and Daniels, '33). The iron retentions of D. B. and D. P. (diet D) were low (0.13 and 0.12 mg. per kilogram) perhaps because they needed less. These two were the only children of the group who came from private homes. Moreover, during the preceding 2 years they frequently had served as subjects for nutrition studies and had received what are believed to be optimum diets.

In the present study, the highest iron retentions found were 0.24 and 0.25 mg. with ingestions of 0.59, 0.65, 0.75 mg. per kilogram respectively, retentions which are no higher than one of those obtained by Leichsenring and Flor at a much

TABLE 3

*Average daily intake, excretion, and retention of iron*

NAME	DATE	WEIGHT	ENERGY INTAKE	IRON INTAKE				IRON EXCRETION				IRON RETENTION			
				Milk	Food	Total	Per kg.	Per 100 Cals.	Urine	Feces	Total	Total	Per kg.		
Diet A. Meat with pasteurized milk															
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
A.C.	2/3	18.0	1677	0.59	10.02	10.61	0.59	0.63	0.29	6.03	6.32	4.29	0.24		
B.C.	2/3	13.5	1272	0.59	8.11	8.70	0.64	0.68	0.33	5.78	6.11	2.59	0.19		
E.I.	2/3	22.6	1974	0.59	12.41	13.00	0.58	0.66	0.34	8.62	8.96	4.04	0.18		
L.T.	3/5	15.0	1613	0.77	8.90	9.67	0.65	0.60	0.39	5.35	5.74	3.93	0.25		
R.H.	3/5	18.1	1628	0.77	9.73	10.50	0.58	0.64	0.55	7.71	8.26	2.24	0.12		
Diet B. Meat with evaporated milk															
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
A.C.	2/11	18.3	1677	0.70	10.58	11.28	0.62	0.67	0.33	8.26	8.59	2.69	0.15		
B.C.	2/11	13.7	1272	0.70	7.52	8.22	0.60	0.65	0.34	5.35	5.69	2.53	0.19		
E.I.	2/11	23.0	1974	0.70	12.70	13.40	0.59	0.68	0.35	9.88	10.23	3.17	0.14		
G.O.	2/25	15.5	1671	1.06	10.58	11.64	0.75	0.70	0.33	7.38	7.71	3.93	0.25		
L.T.	2/25	14.8	1613	1.06	9.73	10.79	0.73	0.67	0.36	7.32	7.68	3.11	0.21		
R.H.	2/25	18.1	1628	1.06	9.34	10.40	0.57	0.64	0.32	7.31	7.63	2.77	0.15		
Diet C. High cereal with pasteurized milk															
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
A.C. <sup>1</sup>	3/27	18.4	1624	0.67	11.58	12.25	0.67	0.75	0.53	7.75	8.28	3.97	0.22		
B.C.	3/27	13.7	1276	0.67	8.84	9.51	0.69	0.75	0.22	6.71	6.93	2.58	0.19		
E.I.	3/27	23.1	1992	0.67	13.48	14.15	0.61	0.71	0.31	9.60	9.91	4.24	0.18		
Diet D. High cereal with evaporated milk															
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
D.B.	4/7	17.0	1453	0.80	9.81	10.61	0.62	0.73	0.33	8.11	8.44	2.17	0.13		
D.P.	4/7	16.4	1494	0.80	9.99	10.79	0.66	0.72	0.45	8.45	8.90	1.89	0.12		
Average		17.4	1614	0.77	10.12	10.89	0.63	0.68	0.35	7.46	7.81	3.08	0.18		

<sup>1</sup> Due to a slight regurgitation on one day, these results are not included in averages.



lower ingestion level (0.40 mg. per kilogram). Perhaps the 50 per cent margin of safety advocated by these authors is not necessary; on the other hand, a depleted child may require considerably more iron than the estimated allowance. But, since in the present study the retentions obtained with diets containing 0.59, 0.65, or 0.75 mg. per kilogram were approximately the same, it would seem that a considerable excess of iron was contained in some of the diets used, and that 0.60 mg. per kilogram should meet the needs for maintenance and growth of the average child of the age studied.

### *Copper*

The daily intake of copper of the children studied varied from 0.069 to 0.113 mg. per kilogram, or from 0.074 to 0.110 mg. per 100 Calories of food ingested. Since the amount of copper needed is not known, an attempt was made to estimate the needs by a study of the ratio of iron to copper in biological materials. As has been suggested, the iron retention may depend on the available copper, or vice versa. The diets fed in the study herein reported contained from seven to nine times as much iron as copper.

The copper excretion in the urine of the children studied was exceedingly small, the amount varying from 0.03 to 0.07 mg. per day. Similar small excretions are reported by Hess, Supplee, and Bellis ('23), who found that the amount excreted in the urine by infants was 0.02 mg. per day, by children from 2 to 3 years of age approximately 0.07 mg., and by adults from 0.09 to 0.12 mg. per day. Rabinowitch ('33), who analyzed urine from fifty adults, obtained values for urinary copper ranging from mere traces to 0.4 mg. per liter. In the present study, there was found to be no outstanding relationship between ingestion and urinary excretion. However, Hess et al. reported that adults on a high copper diet excreted more copper in the urine than on a low copper intake, and Rabinowitch found that urinary copper was increased by administering inorganic copper.

TABLE 4

*Average daily intake, excretion, and retention of copper*

NAME	DATE	WEIGHT	ENERGY INTAKE	COPPER INTAKE			COPPER EXCRETION			COPPER RETENTION			
				Milk	Food	Total	Per kg. Per 100 Cals.	Urine	Feces	Total	Total	Per kg.	
Diet A. Meat with pasteurized milk													
		Kg.	Cals.	Mg	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.		Mg.	Mg.
A.C.	2/3	18.0	1677	0.12	1.11	1.23	0.069	0.074	0.03	0.90	0.93	0.30	0.016
B.C.	2/3	13.5	1272	0.12	0.83	0.95	0.070	0.075	0.03	0.76	0.79	0.16	0.012
E.I.	2/3	22.6	1974	0.12	1.46	1.58	0.070	0.080	0.04	1.01	1.05	0.53	0.023
L.T.	3/5	15.0	1613	0.17	1.21	1.38	0.093	0.086	0.04	0.62	0.66	0.72	0.048
R.H.	3/5	18.1	1628	0.17	1.20	1.37	0.076	0.084	0.04	0.77	0.81	0.56	0.031
Diet B. Meat with evaporated milk													
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.		Mg.	Mg.
A.C.	2/11	18.3	1677	0.30	1.11	1.41	0.077	0.084	0.06	0.91	0.97	0.44	0.024
B.C.	2/11	13.7	1272	0.30	0.90	1.20	0.087	0.094	0.07	0.88	0.95	0.25	0.018
E.I.	2/11	23.0	1974	0.30	1.63	1.93	0.084	0.098	0.04	1.60	1.64	0.29	0.013
G.O.	2/25	15.5	1671	0.36	1.33	1.69	0.109	0.101	0.03	1.18	1.21	0.48	0.030
L.T.	2/25	14.8	1613	0.36	1.33	1.69	0.113	0.105	0.04	1.15	1.19	0.50	0.034
R.H.	2/25	18.1	1628	0.36	1.28	1.64	0.091	0.101	0.03	1.03	1.06	0.58	0.032
Diet C. High cereal with pasteurized milk													
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.		Mg.	Mg.
A.C. <sup>1</sup>	3/27	18.4	1624	0.10	1.68	1.78	0.097	0.110	0.05	0.97	1.02	0.76	0.041
B.C.	3/27	13.7	1276	0.10	1.13	1.23	0.090	0.096	0.04	0.73	0.77	0.46	0.034
E.I.	3/27	23.1	1992	0.10	1.90	2.00	0.087	0.100	0.06	1.33	1.39	0.61	0.026
Diet D. High cereal with evaporated milk													
		Kg.	Cals.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.		Mg.	Mg.
D.B.	4/7	17.0	1453	0.16	1.26	1.42	0.084	0.098	0.04	0.90	0.94	0.48	0.028
	4/7	16.4	1494	0.16	1.28	1.44	0.088	0.096	0.04	1.02	1.06	0.38	0.023
Average		17.4	1614	0.21	1.27	1.48	0.086	0.091	0.04	0.99	1.03	0.45	0.026

<sup>1</sup> Due to a slight regurgitation on one day, these results are not included in averages.

By far the greater proportion of the copper was excreted in the feces, the amount varying from 93 to 98 per cent of the total excretion, values comparable to those obtained for the fecal iron.

The amount of copper retained by the children studied varied from 0.012 to 0.048 mg. per kilogram (average 0.026 mg.), the larger retentions in general being obtained with the diets containing the most copper. For example, B.C. (diet C) received 0.090 mg. and retained 0.034 mg. per kilogram, whereas with ingestions of 0.070 and 0.087 (diets A and B) she retained only 0.012 and 0.018 mg. respectively. Similarly, G. O., L.T., and R.H., with ingestions of 0.109, 0.113, and 0.091 mg. per kilogram (diet B) retained 0.030, 0.034, and 0.032 mg. per kilogram respectively. It would seem, therefore that there was no very large excess in these diets which were presumably high in copper. Indeed, some of the diets may have contained too little.

The amount of copper retained bore no constant relationship to the iron retained, the ratios of the iron-copper retentions varying from 4.7 to 15.8. This would indicate that some children were more depleted in copper, while others were either more depleted in iron or there was an insufficient amount of copper in the diets to meet the needs of the particular child. With the higher copper retentions, there was found to be a closer relationship between iron and copper (ratio 5:1) suggesting that for a given species, there may be a rather definite iron-copper retention ratio.

The highest copper retentions (0.034 and 0.048 mg. per kilogram) were obtained with minimum ingestions of 0.090 and 0.093 mg. per kilogram. Therefore, until further data are available, it is suggested tentatively that diets for children of the preschool age should include not less than 0.100 mg. of copper per kilogram of body weight.

## SUMMARY

Fifteen iron and copper balance studies have been made with eight normal children ranging in age from 4 to 6 years. In a few cases successive studies were made with the same child.

The average iron retention for the group was found to be 0.18 mg. per kilogram, varying from 0.12 to 0.25 mg. with different children. High retentions, often found during first metabolism periods, were interpreted as being due to previous depletion. Diets containing 0.75 mg. per kilogram resulted in no higher retentions than those containing 0.59 or 0.65 mg. It is concluded, therefore, that 0.60 mg. per kilogram will meet the maintenance and growth needs for normal children of the ages studied.

The average retention of copper was found to be 0.026 mg. per kilogram at an average ingestion level of 0.086 mg. per kilogram. But since higher retentions (0.030 to 0.034 mg. per kilogram) were obtained at ingestion levels of 0.090 to 0.093, it is suggested that children of the ages studied should be given no less than 0.100 mg. per kilogram.

## LITERATURE CITED

- BODANSKY, M. 1921 The zinc and copper content of the human brain. *J. Biol. Chem.*, vol. 48, p. 361.
- DANIELS, A. L., M. K. HUTTON, E. KNOTT, G. EVERSON AND O. WRIGHT 1933 Relation of milk ingestion to calcium metabolism in children. *Proc. Soc. Exp. Biol. Med.*, vol. 30, p. 1062.
- ELDEN, C. A., W. M. SPERRY, F. S. ROBSCHT-ROBBINS AND G. H. WHIPPLE 1928 Blood regeneration in severe anemia. XIII. Influence of certain copper salts upon hemoglobin output. *J. Biol. Chem.*, vol. 79, p. 577.
- ELVEHJEM, C. A., AND E. B. HART 1926 Iron in nutrition. II. Quantitative methods for the determination of iron in biological materials. *J. Biol. Chem.*, vol. 67, p. 43.
- ELVEHJEM, C. A., AND C. W. LINDOW 1929 The determination of copper in biological materials. *J. Biol. Chem.*, vol. 81, p. 435.
- ELVEHJEM, C. A., H. STEENBOCK AND E. B. HART 1929 The effect of diet on the copper content of milk. *J. Biol. Chem.*, vol. 83, p. 27.
- GORTER, E., F. GRENDL AND W. A. M. WEYERS 1931 The part copper plays in the anemia of children. *Maandschrift Kindergeneeskunde*, vol. 1, p. 70.

- HART, E. B., H. STEENBOCK, J. WADDELL AND C. A. ELVEHJEM 1928 Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. *J. Biol. Chem.*, vol. 77, p. 797.
- HESS, A. F., G. C. SUPPLEE, AND B. BELLIS 1923 Copper as a constituent in woman's and cow's milk. Its absorption and excretion by the infant. *J. Biol. Chem.*, vol. 57, p. 725.
- KRAUSS, W. E. 1929 Studies on the nutritive value of milk. II. The supplementary value of inorganic iron and copper. *J. Dairy Sci.*, vol. 12, p. 242.
- LEICHSENRING, J. M., AND I. H. FLOR 1932 The iron requirement of the pre-school child. *J. Nutrition*, vol. 5, p. 141.
- LINDOW, C. W., C. A. ELVEHJEM AND W. H. PETERSON 1929 The copper content of plant and animal foods. *J. Biol. Chem.*, vol. 82, p. 465.
- LINDOW, C. W., W. H. PETERSON AND H. STEENBOCK 1929 The copper metabolism of the rat. *J. Biol. Chem.*, vol. 84, p. 419.
- MCHARGUE, J. S., D. J. HEALY AND E. S. HILL 1928 The relation of copper to the hemoglobin content of rat blood. *J. Biol. Chem.*, vol. 78, p. 637.
- MORRISON, D. B., AND T. P. NASH 1930 The copper content of infant livers. *J. Biol. Chem.*, vol. 88, p. 479.
- PETERSON, W. H., AND C. A. ELVEHJEM 1928 The iron content of plant and animal foods. *J. Biol. Chem.*, vol. 78, p. 215.
- QUAM, G. N., AND A. HELLWIG 1928 The copper content of milk. *J. Biol. Chem.*, vol. 78, p. 681.
- RABINOWITCH, I. M. 1933 The copper content of urine of normal individuals. *J. Biol. Chem.*, vol. 100, p. 479.
- REMINGTON, R. E., AND H. E. SHIVER 1930 Iron, copper, and manganese content of some common vegetable foods. *Association of Official Agricultural Chemists*, vol. 13, no. 1, p. 129.
- RICE, F. E., AND J. MISCALL 1923 Copper in dairy products and its solution in milk under various conditions. *J. Dairy Sci.*, vol. 6, p. 261.
- RICE, F. E. 1926 Sweetened condensed milk. VI. Tallowiness. *J. Dairy Sci.*, vol. 9, p. 459.
- ROSE, M. S., E. M. VAHLTEICH, E. ROBB AND E. M. BLOOMFIELD 1930 Iron requirement in early childhood. *J. Nutrition*, vol. 3, p. 229.
- SOBEL, I. P., AND I. J. DREKTER 1933 Determination of the iron content of the blood of children. *Am. J. Dis. Child.*, vol. 45, p. 486.
- STEARNS, G., AND D. L. K. MOORE 1931 Growth in height and weight and retention of nitrogen, calcium, and phosphorus during recovery from severe malnutrition. *Am. J. Dis. Child.*, vol. 42, p. 774.
- SUPPLEE, G. C., AND B. BELLIS 1922 The copper content of cow's milk. *J. Dairy Sci.*, vol. 5, p. 455.
- WANG, CHI CHE, R. KERN AND M. KAUCHER 1929 Metabolism of undernourished children. A study of basal metabolism, caloric balance, and protein metabolism during a period of gain in weight. *Am. J. Dis. Child.*, vol. 38, p. 476.

# THE INFLUENCE OF PREVIOUS DIET, GROWTH AND AGE UPON THE BASAL METABOLISM OF THE RAT

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In addition to the previously reported findings obtained in our comprehensive metabolic study of the rat, considerable data have been collected dealing with the effects of previous diet, growth, age and sex upon the basal heat production of this animal. These data were secured with exactly the same type of rat, under the same laboratory and dietetic conditions (except where specially modified), and with the same metabolism technic as described in our preceding papers (Benedict, '30; Benedict, Horst and Mendel, '32; Horst, Mendel and Benedict, '30, '34 a, '34 b). In some of our experiments it was noted that when the diet of eight rats (about 100 days old) was changed from natural foods to a synthetic diet, that is, a mixture of purified food materials, the basal metabolism decreased. Observations with older rats were therefore made, to compare further the metabolic effects of natural and synthetic foods. Another phase of our study of the influence of diet upon metabolism dealt with the effects of different levels of protein in the diet, the maximum percentage being limited to an amount that would not produce hypertrophy of the kidneys (Osborne, Mendel, Park and Winternitz, '27). The differences in the rates of growth of our rats, dependent upon dietetic conditions, resulted in,

1) animals that may properly be designated as 'rapid growth' rats (average daily gain in weight about 4 gm.) and, 2) 'slow growth' rats (average daily gain in weight about 2 gm.). The basal metabolism of these animals was compared at different ages throughout the first year of life. In some young and half-grown rats, growth was deliberately retarded by feeding a 'normal' diet insufficient in quantity. Our measurements of the metabolism of these 'stunted' rats enable a comparison with the metabolism of normal rats of the same age but larger weights and of the same weight but younger ages. In some of the stunted rats the metabolism during 'resumed' growth on full rations was likewise studied. Finally, data were obtained with both male and female rats that contribute information regarding the influence of old age on the metabolism. This factor of old age has been too little studied, partly because animals, after they have reached the adult stage, are rarely maintained in the laboratory for the specific purpose of noting the influence of age. The increased interest in the physiology of old age, stimulated by Sherman's discovery that the average length of life of rats can be increased 10 per cent by dietetic devices (Sherman and Campbell, '28, '30), makes any contribution to this question of old age of great importance. Although the majority of our measurements were made on male rats, enough were made on females, particularly in studying the age factor, to contribute some information regarding the influence of sex.

### *Natural versus synthetic foods*

The synthetic diet used was a mixture of purified food materials<sup>1</sup> supplemented daily by 300 mg. dry brewers' yeast and 150 mg. cod liver oil. The natural diet consisted of dog biscuit, milk food,<sup>2</sup> and fresh lettuce. Six male rats, litter-mates, were fed the synthetic diet for from 6 to 7 weeks (initial age 80 days). Their basal metabolism was measured twice during this dietary regimen, viz., in the fourth and the

<sup>1</sup> The 'medium-protein' diet described on page 143.

<sup>2</sup> Whole milk powder, 60 per cent; cornstarch, 12 per cent; lard, 28 per cent.

seventh weeks. The natural diet was next fed, and the metabolism was determined under basal conditions after the rats had been 2 and 4 weeks, respectively, on this regimen. In all instances the animals were measured at 30°C., had been 24 hours without food, and (as was customary in all of our observations on rats) had lived at 28°C. for 24 hours before the experiments. We were dealing, therefore, not with the immediate after-effect of food but with the problem as to whether the different diets caused differences in body com-

TABLE 1

*Basal metabolism of rats receiving synthetic and natural foods. (Average values)*

FOOD	NUMBER OF RATS STUDIED	AGE	WEIGHT	ACTIVITY	HEAT PRODUCTION PER SQUARE METER <sup>1</sup> PER 24 HOURS		
					Minimum	Maximum	Average
		<i>days</i>	<i>gm.</i>	<i>p ct.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>
Synthetic	6	109	276	14	649	796	719
Synthetic	6	126	273	16	596	730	679
Natural	6	140	305	18	707	895 <sup>2</sup>	789
Natural	6	154	327	27	755	952 <sup>2</sup>	868
Synthetic	3	166	335	18	609	729	684
Synthetic	2	194	341	21	635	740	688
Natural	2	168	354	15	689	721	705
Natural	3	193	371	14	706	781	748

<sup>1</sup>  $S = 9.1 \times w^{2/3}$

<sup>2</sup> Active more than 20 per cent of period of measurement.

position that were reflected in the metabolism. The average results of this study are recorded in the upper half of table 1.

If the slight difference in age is disregarded, it would appear from these data that the diet of natural foods resulted in an increased metabolism. Thus the minimum values when the rats were on the synthetic diet are lower than the minimum values when they were on a natural diet. The comparison of the maximum values is complicated by the relatively excessive activity of the rats when receiving natural foods. These rats were seldom altogether quiet, i.e., there were continual slight



movements, whereas the rats on the synthetic diet often showed periods of complete quiescence alternated with periods of great restlessness. At 140 days with natural foods, the maximum heat production, unaffected by great activity, was 801 Calories or considerably higher than the maximum heat production of 730 Calories at 126 days with the synthetic diet. At 154 days, during the period of natural foods, all of the results except the minimum value of 755 Calories denote metabolism with high activities. The average heat production was lower during the period of the synthetic diet than during the period of natural foods. The grand average for the two series with the synthetic diet was 699 Calories as compared with the grand average of 829 Calories with the natural diet. Supplementary evidence secured with twenty-four other male rats from 91 to 196 days old that had been given a diet of natural foods for at least 2 weeks showed a uniformly high basal metabolism averaging 778 Calories per square meter of body surface (average activity 13 per cent). Another group of twenty-five males of the same ages, subsisting upon a synthetic diet, gave low values averaging 720 Calories (average activity 11 per cent). Differences in activity did not account for the difference in metabolic levels, for even with the factor of excessive activity eliminated (as is the case in the average values of 778 and 720 Calories cited above), the metabolism of the rats that had been receiving the natural foods was 8 per cent higher than that of the rats subsisting upon the synthetic diet. The picture is complicated, however, by the fact that with the six rats used for the special study the body weights remained practically unchanged during the period of feeding with the synthetic diet whereas they increased during the period on natural foods.

When the six rats in the special study were 154 days old, three of them were again given a synthetic diet and the other three continued to live on natural foods. Again two metabolism observations were made on each rat, at least 3 weeks apart and 2 weeks after the dietary alteration. The results obtained are recorded in the lower half of table 1. Although

too few animals were studied in this series to permit drawing any definite conclusions with regard to whether there is any sustained after-effect of a continued diet of natural foods, it would appear that, in general, the previous diet did not play a significant role in the metabolism of these rats.

### *The protein factor*

In man and dogs the specific dynamic action of protein is higher than that of any other foodstuff. The pronounced immediate after-effect of protein has led to the general belief that rations rich in protein result in a high basal metabolism.

TABLE 2  
*Composition of diets*

COMPONENTS	HIGH PROTEIN	MEDIUM PROTEIN	LOW PROTEIN
	<i>p ct.</i> <sup>1</sup>	<i>p.ct.</i> <sup>1</sup>	<i>p.ct.</i> <sup>1</sup>
Casein <sup>2</sup>	60	30	6
Cornstarch	12	42	65.7
Butter fat	9	9	9
Lard	15	15	15
Salt mixture <sup>3</sup>	4	4	4
Cystine	0	0	0.3

<sup>1</sup> By weight.

<sup>2</sup> On an air-dry basis, the casein contained 13.3 per cent nitrogen.

<sup>3</sup> Described by Osborne and Mendel ('19).

Wang ('30) and her associates did not find any essential difference in the basal metabolism of normal women with protein intakes varying from 0.6 to 2 gm. per kilogram of body weight. The data of Hogan and Pilcher ('30) do not show any significant differences in the metabolism of rats with diets containing 12 and 24 per cent of casein. In our investigations sixteen male rats were used to study the effects of high-, medium- and low-protein diets, there being five rats in each of the first two groups and six in the low-protein group. These rats were from 90 to 120 days old at the inception of the protein regimen. Littermates were chosen in groups of three, one for each diet. The diets (table 2) were

so compounded that, although the quantity of casein varied, the total energy value remained 5.2 Calories per gram. Each rat received daily 300 mg. dry brewers' yeast and 145 mg. cod liver oil. The basal diets were fed ad libitum. The daily nitrogen consumption, calculated per 200 gm. of rat, averaged 494, 262 and 54 mg. in the high-, medium- and low-protein groups, respectively. Thus the rats in the low-protein group received less nitrogen than the minimum requirement for rats of 200 to 350 gm. observed by Osborne and Mendel ('15). Strict comparisons are not possible, however. In the older Osborne and Mendel experiments the nitrogen content of the protein-free milk was disregarded. On the other hand, the supplementary cystine was not used in their diets.

TABLE 3  
*Basal metabolism of rats on different protein levels*

PROTEIN LEVEL	NUMBER OF RATS STUDIED	AGE		WEIGHT		HEAT PRODUCTION PER SQUARE METER PER 24 HOURS		
		Initial	Final	Initial	Final	Minimum	Maximum	Average
		<i>days</i>	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>
High	5	116	140	261	285	686	773	724
Medium	5	119	145	252	271	685	766	729
Low	6	118	145	249	247	639	719	677

The basal metabolism of each rat was determined at three different times, once when the rats had been on the special protein diets for about 2 weeks, and twice thereafter at intervals of about 2 weeks. The values observed in the three determinations on each rat were fairly constant. The results, recorded as the average heat production and the range observed for the rats in each dietary group, are shown in table 3. The initial ages and weights are those noted at the time of the first metabolism measurement, and not at the start of the feeding on the special diets. The values for the average metabolism and for the range show that there was practically no difference in the metabolism of the rats in the high- and medium-protein groups, but a definitely lower metabolism in the animals in the low-protein group. The rats in the latter

group likewise had a somewhat lower final weight. From the post-mortem examinations and from this finding of a low metabolism with the rats on the low-protein diet it can be concluded that deleterious physiological effects were beginning to appear in the rats on the extremely low protein diet.

### *Suppression of growth or stunting*

In studies of the laws governing the growth of the rat, various methods (so-called 'stunting processes') of maintaining the body weight of the youthful rat at a constant level have been developed. The fact that during this stunting process the animal retains approximately its normal configuration without appearance of extreme emaciation removes the problem of the metabolic effect of suppression of growth completely from the well-known problem of undernutrition. In studies on undernutrition adult animals and humans have been made to lose a considerable proportion of their previously acquired body weight. In our investigation, on the contrary, the growth of the rat was suppressed by feeding insufficient quantities of a normal diet.<sup>3</sup> In one series, four male rats (about 31 days old at the start) received limited quantities of food for 42 days. Metabolism was measured four times, initially on the tenth day of experimental feeding and three times thereafter at intervals of about 10 days. The results are recorded in table 4, from which it can be seen that for 31 days the body weights of these rats remained almost stationary. The heat production also remained essentially constant, although with all four animals it was somewhat lower at the end of the series than at the start. The activity varied somewhat, but for the most part permitted comparison of the various values.

On the average the metabolism decreased from 708 Calories per square meter of body surface on the tenth day of stunting to 599 Calories on the thirty-first and the thirty-fourth days of stunting—a decrease of 15 per cent. During these

<sup>3</sup> The diets used in our experiments were similar to the 'low calorie' diets described by Winters, Smith and Mendel ('27).

24 days the rats had increased in age from about 40 to 65 days. At corresponding ages, the metabolism of seven normal male rats averaged 868 Calories (at 42 to 45 days) and 828 Calories (at 56 to 70 days) per square meter of body surface (table 8, p. 152). Hence, the heat production of the stunted

TABLE 4

*Basal metabolism of male rats stunted by restriction of Calorie intake. (Measured at 30°C. and 18 to 24 hours after food)*

RAT NO. <sup>1</sup>	AGE	WEIGHT	DAYS OF STUNTING	ACTIVITY	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per square meter
	<i>days</i>	<i>gm.</i>		<i>p.ct.</i>	<i>Cal.</i>	<i>Cal.</i>
1	45	56	10	5	33.6	707
	66	62	31	10	28.1	608
	73	63	38	6	30.8	674
	76	67	41	..	28.7	640
2	41	61	10	15	31.5	681
	50	64	19	7	29.7	651
	62	65	31	7	24.3	537
	72	67	41	10	28.7	640
3	41	57	10	15	33.3	704
	62	63	31	8	28.9	632
	65	65	34	13	27.7	612
	72	67	41	6	29.0	647
4	44	62	13	11	34.1	739
	62	63	31	19	30.5	667
	65	65	34	25	28.9	639
	72	67	41	17	30.7	687

<sup>1</sup> In this report and in our preceding reports the rats have been numbered consecutively, beginning with no. 1, for purposes of ready reference. The numbering has been done independently in each report, however, and rat 1 or rat 24, for example, in this article is not the same as rat 1 or rat 24 in our earlier papers.

rats was 18 per cent below normal on the tenth day of stunting (41 to 45 days old) and 28 per cent lower than normal on the thirty-first and thirty-fourth days of reduced food consumption. On the forty-first day of stunting the heat production averaged 654 Calories. At this time the stunted rats were 72 to 76 days old and weighed 67 gm. each. Twelve normal

males of about the same age but not of the same weight had an average basal heat production of 785 Calories per square meter of body surface (table 8, p. 152). Thus, on the basis of equal age, the average metabolism of the four stunted rats was 17 per cent below that of the normal rats. The metabolism of five normal males of about the same weight (69 gm.) as the 72-day-old stunted rats but much younger was, on the average, 834 Calories (unpublished data). Thus the metabolism of the stunted rats was 22 per cent below that of normal rats of the same weight.

In a second series of observations the basal metabolism of two male rats (nos. 5 and 6, table 5) was studied during 84 to 89 days of stunting. The metabolism decreased during the first 28 days, remained at about the same level for the next 7 days, and increased as the stunting period was prolonged. When these rats were 52 and 64 days old, respectively, and had been 28 days on the restricted diet, their heat production averaged 598 Calories or practically the same as the average of 599 Calories noted with the four rats in the first series with 31 to 34 days of reduced rations. On the eighty-fourth and eighty-ninth days of stunting the metabolism was 572 and 693 Calories, respectively. The greater activity of rat 6 at this time undoubtedly explains the difference observed in the metabolism of the two rats. The average metabolism of seven normal males from 101 to 126 days old or approximately the same age as these two stunted rats at the close of the period of reduced food intake was 761 Calories per square meter of body surface (table 8, p. 152). In all these studies there were at times unavoidable differences in activity. Indeed, the stunted animals, as seen from table 5, were unusually active during measurements. Nevertheless, the heat production was unquestionably lower in the stunted rats, in spite of their great activity, than in normal rats of the same age and likewise lower than in normal rats of the same weight.

At the end of the experiments the four stunted rats in the first series (table 4) were killed and their bodies analyzed for fat. The bodies of two normal rats of the same age but

weighing 128 and 137 gm., were likewise analyzed. The total fat in the stunted rats was 5.5, 1.1, 1.6 and 0.7 per cent of the total body weight as compared with 5.5 per cent in the two normal rats. The subcutaneous fat of three of the stunted rats averaged 0.7 per cent of the total body weight (for rat 1

TABLE 5

*Basal metabolism of male rats stunted by restriction of Calorie intake. (Two rats in one chamber, measured at 28°C. and 24 hours after food)*

RAT NO.	AGE	WEIGHT	DAYS OF STUNTING	ACTIVITY	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per square meter
	<i>days</i>	<i>gm.</i>		<i>p.ct.</i>	<i>Cal.</i>	<i>Cal.</i>
5	40	55	4	..	35.0	731
6	28	55				
5	44	60	8	37 <sup>1</sup>	32.3	681
6	32	54				
5	51	58	15	32 <sup>1</sup>	30.7	654
6	39	56				
5	55	56	19	31 <sup>1</sup>	30.2	643
6	43	58				
5	64	60	28	21 <sup>1</sup>	28.0	598
6	52	58				
5	71	58	35	19 <sup>1</sup>	28.9	618
6	59	58				
5	82	60	46	35 <sup>1</sup>	31.3	671
6	70	60				
5	89	60	53	46 <sup>1</sup>	30.7	657
6	77	60				
5	97	59	61	60 <sup>1</sup>	34.8	741
6	85	58				
5	107	61	71	16	31.5	681
	114	63	78	8	28.9	632
	120	68	84	10	25.6	572
6	98	61	74	40	32.8	709
	106	65	82	14	33.8	748
	113	69	89	18	30.7	693

<sup>1</sup> On the basis of the total activity of the two rats.

it amounted to 3.8 per cent). The subcutaneous fat of the normal rats amounted to 3 per cent. Thus the normal rats were better protected against low external temperatures than the stunted rats.<sup>4</sup>

### *Resumed growth after stunting*

It is a known fact that rats whose growth has been retarded for some time will gain regularly in body weight with resumed liberal feeding. With such rats it is possible to study the metabolism during growth at an age when growth has normally ceased. Five male rats (114 to 135 days old) that had been living on inadequate rations (deficient in various essential factors) for from 73 to 98 days were subsequently given a diet of natural foods. The metabolism was measured at the end of the period of retarded growth and on three different occasions during the period of resumed growth, as shown in table 6. In all determinations the activity was less than 15 per cent. The heat production at the end of the period on inadequate diets averaged 706 Calories per square meter of body surface, or somewhat lower than the value, 761 Calories (table 8, p. 152), for seven normal male rats of about the same age. The metabolism apparently did not increase immediately after the diet was changed, for the heat production of rat 7 per unit of weight and of body surface was somewhat lower 3 days after realimentation than it was during the period of suppressed growth. On the seventh to the tenth days of resumed growth, the heat production per square meter of body surface was 9, 11 and 14 per cent higher than the metabolism with inadequate diets (rats 8 to 10). By the eighteenth day of realimentation, the metabolism per unit of surface area had reached a maximum, being 33 and 15 per cent above the level at the close of the stunting period (rats 7 and 8). Thereafter, the metabolism per unit of surface area gradually decreased, varying somewhat with the individual rat. In the case of rats 7 and 9 the experiments were con-

<sup>4</sup> We are indebted to Dr. L. L. Reed of Yale University for the fat determinations.



tinued for 75 and 77 days. At the close of the study these rats weighed 406 and 354 gm., respectively, weights not much different from the average body weights of 429 gm. (table 7, p. 152 and 341 gm. (table 8, p. 152) for normal rats of about the same age. Likewise, the heat production of rats 7 and 9, 707 and 664 Calories, was not very far from the average metabolism of normal rats of the same age, 755 Calories being observed on eleven rapid growth rats (table 7) and 714 Calories on eight slow growth rats (table 8). It would thus appear that with realimentation the stunted rat not only may resume its normal growth unhampered but that its metabolic level when normal growth is attained will be not far from that of the normally growing rat of the same age and weight.

TABLE 6

*Basal metabolism of male rats in relation to resumption of growth after retardation. (Measured at 30°C. and 24 hours after food)*

RAT NO.	PERIOD OF GROWTH	AGE	WEIGHT	DAYS OF RESUMED GROWTH	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per square meter
		<i>days</i>	<i>gm.</i>		<i>Cal.</i>	<i>Cal.</i>
7	Retarded	118	129	..	23.7	670
	Resumed	121	142	3	22.1	633
		135	239	17	26.2	892
		170	345	52	20.5	788
		193	406	75	17.4	707
8	Retarded	119	71	..	32.9	750
	Resumed	126	107	7	31.2	815
		137	143	18	30.1	863
		161	230	42	25.0	842
9	Retarded	114	109	..	25.4	663
	Resumed	121	150	7	26.0	759
		167	305	53	18.7	692
		191	354	77	17.1	664
10	Retarded	135	119	..	27.5	741
	Resumed	145	172	10	26.8	822
		166	229	31	24.2	812
11	Resumed	112	118	16	31.4	845
		161	273	65	23.8	846

Even after stunting, therefore, rats may be made to grow and reach a size approximately normal for albino rats of similar strains. As can be seen from table 6, these rats had a remarkably rapid growth after liberal feeding, the most striking case being that of rat 7, which between the ages of 118 and 135 days, that is, during 17 days of realimentation, increased in weight from 129 to 239 gm. or at the rate of more than 6 gm. per day. Hence it is clear that the problem of resumed growth and metabolism is closely bound up with the question of rate of growth.

### *Rate of growth*

The usual rate of growth of the normal male rat between the ages of 30 and 90 days is about 2.3 gm. per day (Donaldson, '24), but with special diets much more rapid rates of growth are readily secured. Our experiments included observations on a group of rats designedly fed so that they gained weight at the rate of 4 gm. or more per day between the ages of 30 and 90 days. These were our so-called 'rapid growth' rats (table 7). These rats received a synthetic food mixture<sup>5</sup> with daily supplements of 20 gm. of fresh lettuce and 200 to 400 mg. dry brewers' yeast. Another group of rats designated 'slow growth' (table 8) were fed our standard synthetic diet<sup>6</sup> with quantities of yeast limited to obtain the desired gain of about 2 gm. a day. Initially, 100 to 200 mg. of yeast were fed daily. This was gradually increased to 300 and 400 mg. In comparing these two groups of rats, we have to deal with the fact that the rapid growth rats were much heavier than the slow growth rats at the same ages, and again we are confronted by the problem as to the best physiological method of comparing the metabolism of animals of different sizes. If nothing more than for the purpose of showing the extreme complexity of a problem that has hitherto been assumed very simple, we have retained the body-surface comparison in the heat values recorded in tables 7 and 8. In all

<sup>5</sup> Described by Osborne and Mendel ('26).

<sup>6</sup> Described by Horst, Mendel and Benedict ('34 a).

instances the rats were active for less than 18 per cent of the period of measurement and usually less than 10 per cent.

TABLE 7

*Basal metabolism of 'rapid growth' rats. (Males that gained 4 gm. daily between the ages of 30 and 90 days; measured at 30°C. and 24 hours after food)*

NUMBER OF RATS	AGE RANGE	WEIGHT		AVERAGE ACTIVITY	HEAT PRODUCTION PER 24 HOURS		
		Range	Average		Average per 200 gm.	Per square meter	
						Range	Average
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>p.ct.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>
14	42- 55	94-139	123	10	33.8	766-1041	923
13	56- 69	122-198	154	11	28.9	776- 997	849
11	71- 85	196-321	230	10	24.2	752- 891	810
5	88- 99	246-275	262	11	23.8	787- 895	836
6	101-130	283-348	321	11	19.3	676- 799	725
10	136-159	332-500	393	11	18.6	653- 882	745
11	162-224	354-487	429	10	18.2	643- 854	755
14	233-294	433-664	521	8	15.9	620- 800	701

TABLE 8

*Basal metabolism of 'slow growth' rats. (Males that gained 2 gm. daily between the ages of 30 and 90 days; measured at 30°C. and 24 hours after food)*

NUMBER OF RATS	AGE RANGE	WEIGHT		AVERAGE ACTIVITY	HEAT PRODUCTION PER 24 HOURS		
		Range	Average		Average per 200 gm.	Per square meter	
						Range	Average
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>p.ct.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>
7	42- 45	70- 98	83	7	36.5	798-948	868
11	56- 70	100-138	119	8	30.6	727-909	828
12	71- 85	120-171	140	9	27.6	701-845	785
14	87- 99	150-188	175	11	25.8	708-952	792
7	101-126	166-242	218	8	23.2	611-815	761
17	131-148	224-281	248	10	21.3	646-851	734
8	170-226	312-364	341	9	18.6	587-833	714
13	232-302	307-402	359	11	18.8	687-818	734

Fourteen 'rapid growth' rats at 42 to 55 days of age and weighing from 94 to 139 gm. had an average heat production of 33.8 Calories per 200 gm. of body weight and 923 Calories per square meter of body surface. Seven 'slow growth' rats

from 42 to 45 days of age but weighing 40 gm. less on the average than the rapid growth rats showed average heat values per unit of weight and body surface of 36.5 and 868 Calories, respectively. Here the picture is exactly the opposite on the two bases of comparison, namely, the slow growth rats had a lower heat production than the rapid growth rats per unit of surface area and a higher heat production per unit of weight. The heat values per 200 gm. of weight are in full conformity, however, with the general finding that animals of the same species usually have a larger heat production per unit of weight, the smaller the animal.

At 56 to 69 days of age thirteen rapid growth rats had an average heat production of 849 Calories per square meter of body surface, whereas eleven slow growth rats of the same age had an average metabolism of 828 Calories. Thus, on this basis, the slow growth rats had a slightly lower metabolism than the rapid growth rats. Per unit of weight, however, the slow growth rats had a higher heat production than the rapid growth rats, the average values being 30.6 and 28.9 Calories, respectively. At these ages the slow growth rats weighed 35 gm. less, on the average, than the rapid growth rats. At 71 to 85 days of age eleven rapid growth rats had an average metabolism of 810 Calories and twelve slow growth rats of the same age, 785 Calories. The body weights of the slow growth animals were 90 gm. less, on the average, than those of the rapid growth animals, and the heat production per 200 gm. of weight was higher, 27.6 as compared with 24.2 Calories.

From 88 to 99 days of age, the metabolism of five rapid growth rats averaged 836 Calories per square meter of body surface; that of fourteen slow growth rats, 792 Calories. Hence, on the body surface basis, the metabolism of the rapid growth rats was still definitely higher than that of the slow growth rats. Per unit of weight, however, the average metabolism of the rapid growth rats, 23.8 Calories, was lower than the average heat production of the slow growth rats, 25.8 Calories. At these ages, the slow growth rats weighed 87 gm. less, on the average, than the rapid growth rats.

At 88 to 99 days the metabolism (per unit of surface area) of both the rapid growth and the slow growth rats was somewhat higher than that at 71 to 85 days of age. As the average age of sexual maturity of male rats has been found to be 109 days (Slonaker and Card, '23), the higher metabolism of our rats at 88 to 99 days suggests a prepubertal rise similar to that observed in boys by DuBois ('16) and recently confirmed by Topper and Mulier ('32) and Bruen ('33). The Nutrition Laboratory did not find this rise with girls (Benedict and Hendry, '21).

In general, comparisons on the body surface basis show that the metabolism of the rapid growth rats from 40 to 100 days of age (the period during which growth proceeded at the rate of 4 gm. a day) was higher than that of the slow growth rats. From 101 to 226 days of age, however, the metabolism of the two groups did not differ appreciably. From 233 to 294 days of age, the rapid growth rats had a slightly lower metabolism than the slow growth animals. At this age the rapid growth rats weighed from 433 to 664 gm. and the slow growth rats from 307 to 402 gm.

The most striking metabolic differences between the two groups of rats appear in the early stages of life, when the factors of rate of growth and age are most prominent. The picture of higher metabolism in the rapid growth rats per square meter of body surface is offset in large part by the finding that per 200 gm. of body weight their metabolism is the same or slightly lower than that of the slow growth rats. In the light of the comparisons at the older ages it is probably reasonable to conclude that the rate of growth is practically without effect upon the basal metabolism.

### *Metabolism in old age*

In addition to our observations on the metabolism during the period of growth, when there are simultaneously changes in body weight and in age, we had occasion to study the metabolism of a few male and female rats in old age. These

animals<sup>7</sup> received a stock diet of dog biscuit and wheat germ food<sup>8</sup> which they ate ad libitum. The adjuvants, fresh lettuce or cabbage, were fed twice each week.

Six of our male rats (nos. 12 to 17, table 9) were allowed to live far beyond the usual time allotted to rats in laboratory studies. Four of these showed increases in weight during the period of observation and two showed no increases. The important feature of the study, however, is that the metabolism measurements on these animals were continued into the third year of life. Although there were considerable fluctuations in the metabolism of the rats recorded in table 9, especially in the last months, in general one can conclude that the metabolism of the male rat remains at a practically constant level during the second and third years of life. This conclusion is based upon experiments with a small number of rats, and further data are needed. Moreover, the situation is somewhat complicated by the fact that these rats were for the most part unusually large. Three certainly were extremely large, and the other three were well over the average weight for the normal adult male rat. It is unfortunate that in the study of this particular problem the same rat was not observed over a longer period of time, although the measurements on two of these animals (nos. 12 and 17) did extend over nearly a year.

Fourteen female rats (nos. 18 to 31) were studied during the second and third years of life. The ages of rats 28 to 31 ranged from about 300 or 450 to 800 or 900 days. The weights of nine of the rats (nos. 18 to 26) ranged from 218 to 299 gm. Five of the rats (nos. 27 to 31) weighed over 300 gm. In fact, rat 30 weighed nearly 500 gm. toward the end of the period of study—an extremely heavy weight for a female rat. The heat production per square meter of body surface (table 10) was in the majority of instances materially

<sup>7</sup> Rats 15 and 16 were 'rapid growth' rats and received the diet explained on page 151 until they were about 870 days of age. Thereafter, they were fed the stock diet.

<sup>8</sup> Whole milk powder 50 per cent, wheat germ 20 per cent, lard 30 per cent.

under 670 Calories, indeed often under 600 Calories. If allowances are made for differences in activity (allowances

TABLE 9

*Basal metabolism of male rats in the second and third years of life. (Measured at 28° to 30°C. and 24 hours after food)*

RAT NO.	AGE	WEIGHT	RECTAL TEMPERATURE	ACTIVITY	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per square meter
	<i>days</i>	<i>gm.</i>	<i>°C.</i>	<i>p.ct.</i>	<i>Cal.</i>	<i>Cal.</i>
12	393	517	37.4	..	16.2	713
	459	553	37.0	..	15.1	681
	566	554	37.8	..	12.8	576
	601	543	37.8	..	16.5	737
	646	545	38.1	6	14.0	628
	709	504	....	..	14.7	643
13	467	567	37.2	7	16.5	750
	514	591	37.1	5	14.9	686
	645	602	37.2	..	14.8	687
	659	594	37.8	..	15.5	717
	746	660	37.3	..	16.3	781
	774	600	38.2	..	17.9	831
14	764	345	37.8	12	17.8	688
	780	348	38.2	23	18.2	702
	883	321	37.6	4	20.0	749
15	807	405	36.4	21	19.1	777
	828	414	35.6	..	17.4	711
	835	416	37.2	..	17.5	720
	889	430	35.9	..	18.7	776
	896	429	36.0	23	20.3	840
16	804	344	37.3	21	19.0	729
	832	331	36.6	3	18.1	690
	840	343	36.8	13	16.3	628
	879	348	35.6	..	19.8	767
	889	374	35.9	8	17.5	695
	969	447	37.4	14	20.0	840
	1060	400	36.2	7	18.1	735
17	443	500	36.7	8	16.1	702
	513	546	37.1	..	14.1	632
	649	540	37.8	..	15.5	694
	684	558	37.6	13	13.6	615
	759	547	38.0	..	14.2	639

made only with difficulty), it is evident that, in general, the metabolism of these female rats was not materially altered during the second and third years of life. Indeed, compared with females at ages between 62 and 200 days, the metabolism was lower during the second and third years of life. These results accord with the results obtained on the male rats (table 9), although the metabolism of the males per unit of surface area was somewhat higher, on the whole, than that of the females of similar ages.

The rectal temperatures of these male and female rats, obtained at the time of the metabolism measurements, are also recorded in tables 9 and 10. Although we recognize that there are not enough temperature records to permit making any deductions, we have included the data in the tables simply as a general contribution regarding the physiology of old age.

The age factor with female rats was of unusual interest to us, in view of the finding of Benedict and MacLeod ('29) that, contrary to practically all animals thus far studied by the Nutrition Laboratory, female rats of the colony raised at Columbia University had unquestionably an increasing metabolism per unit of surface area with advancing age. This trend is so clearly shown by the chart published by these investigators that there is little doubt as to its probability. Male rats, much fewer in number, studied at that same time, gave by no means the same clear picture of increasing metabolism with advancing age, but certainly showed no tendency for a decrease in metabolism with age. The results with the Yale female rats are entirely the opposite of those noted with the Columbia female rats. Careful analysis of the data indicates that after about the first year the weight of the Columbia rats remained fairly constant, whereas that of the Yale rats continued to increase, certainly until they were at least 500 days old. Indeed, the Yale rats of both sexes weighed, on the average, from 70 to 75 per cent more, in the second and third years of life, than did the Columbia rats. Combined with the factor of increasing age, therefore, there are the factors of continually increasing body weight and of markedly



TABLE 10

*Basal metabolism of female rats in the second and third years of life.<sup>1</sup> (Measured at 30°C. and 24 hours after food)*

RAT NO.	AGE	WEIGHT	RECTAL TEMPERATURE	ACTIVITY	HEAT PRODUCTION PER 24 HOURS		RAT NO.	AGE	WEIGHT	RECTAL TEMPERATURE	ACTIVITY	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per sq.m.						Per 200 gm.	Per sq.m.
	days	gm.	°C.	p.ct.	Cal.	Cal.		days	gm.	°C.	p.ct.	Cal.	Cal.
18	436	299	36.9	12	19.6	722	28	274	235	38.7	8	20.2	686
	455	297	38.0	3	18.2	667		288	238	37.4	..	22.7	774
19	494	249	35.9	..	18.2	631		440	282	37.0	8	20.7	746
	517	250	35.1	..	19.0	657		494	284	38.7	10	18.0	651
								531	297	39.1	9	16.3	597
20	493	270	37.2	2	17.3	616		595	308	38.7	2	17.0	632
	496	271	37.3	12	17.3	617	29	658	314	38.3	5	16.6	620
	573	288	37.8	5	19.2	698		721	315	38.1	9	18.4	688
	580	293	37.2	4	17.6	643		763	330	38.4	6	17.2	652
21	493	239	36.7	11	19.5	666		838	324	37.6	..	17.9	675
	496	238	37.3	8	18.0	613	30	454	344	38.6	3	14.7	565
	573	244	37.6	4	20.1	690		503	336	38.7	14	15.6	597
	580	250	36.3	6	18.6	643		551	396	38.3	6	15.7	633
22	516	288	37.2	5	16.3	589		604	385	39.0	4	13.2	528
	525	285	37.4	7	15.4	558		650	389	38.2	5	13.9	557
							31	730	374	37.3	12	16.2	643
23	559	218	37.8	8	23.4	773		792	363	37.8	..	17.2	675
								867	356	36.7	..	16.2	631
24	530	234	....	..	21.2	717		453	334	37.9	2	16.8	642
	550	242	36.4	9	21.0	720	30	502	347	37.3	11	15.0	579
								628	382	38.8	9	16.0	637
25	550	293	36.4	..	16.8	613		656	410	38.3	13	16.3	664
	573	271	35.7	..	18.4	654		726	484	38.3	..	14.3	617
26	567	267	36.2	..	20.4	724		757	488	38.4	21	14.9	643
	590	263	35.8	..	23.3	821	31	832	480	37.7	27	15.7	674
								856	458	38.6	18	15.5	658
27	536	310	37.6	8	17.0	628		895	448	36.7	4	16.1	678
	547	323	38.4	6	16.4	617		580	375	37.2	6	15.9	632
	733	339	37.4	24	19.0	714		622	362	38.3	20	17.7	692
	742	331	38.1	12	17.0	646		674	373	38.1	9	15.9	631
								627	382	37.8	16	14.4	573
							31	758	375	38.3	..	16.3	646
								807	376	37.8	8	15.0	593
								856	373	37.2	16	17.0	675
								888	386	37.0	14	17.2	687
								973	397	37.8	..	18.3	738

<sup>1</sup> Previously mated but studied when not pregnant and not lactating.

different body weights for age with the two groups. Thus in this comparison we encounter again the as yet wholly unsolved problem as to how the heat values can be expressed to rule out differences in size. It is clear that calculations per unit of body weight do not eliminate this factor, and it is becoming increasingly evident that there is no correction for this factor in the calculations per unit of surface area. With unusually large rats we found that the heat production per square meter of surface area was smaller, the larger the rat (Benedict, Horst and Mendel, '32). Comparison of the heat values on this same basis for the Columbia and Yale female rats substantiates this finding, the lighter weight Columbia rats having a higher metabolism per unit of surface area in the second and third years of life than the heavier Yale rats. If there were any method of calculating the heat values to correct for differences in size (and thus far we have been unable to find any satisfactory substitute for the traditional calculations per unit of weight and surface area), it is conceivable that the heat production of the Columbia rats,<sup>9</sup> instead of increasing with advancing age, might show the same tendency as did that of the Yale rats to remain unaltered at the older ages.

#### SUMMARY

The basal metabolism of male rats (91 to 196 days old) that received a diet of natural foods was somewhat higher initially (2 weeks after the diet was changed) than the metabolism of other male rats on a so-called synthetic diet. When these diets were fed to rats for a continued length of time, however, they played no significant role in the metabolism.

There was practically no difference in the basal metabolism of rats fed diets of high-protein and medium-protein content,

<sup>9</sup> These earlier results were obtained before the importance was recognized of keeping the animals at 28°C. for 24 hours before the metabolism measurements. In the few instances where female rats of the Columbia series were measured at 28° after living for 24 hours at this same temperature, it was noted that the metabolism did not increase with old age. Further experiments on this particular problem are at present in progress with the Columbia series of rats.

but rats on a low-protein diet had a lower metabolism and in these rats post-mortem examinations showed that the diet had begun to have a deleterious physiological effect.

The basal heat production of young rats stunted in growth was lower than that of normal rats of the same age but larger weight and lower than that of normal rats of the same weight but different age. With realimentation the stunted rat not only resumed its normal growth but its basal metabolism, when normal growth was attained, was at approximately the same level as that of the normally growing rat of the same age and weight.

The rate of growth, whether slow or rapid, did not have any appreciable effect upon the basal metabolism of normal male rats. However, from 40 to 100 days of age the heat production per unit of surface area of the rapidly growing rats was somewhat higher than the metabolism of the slowly growing rats, whereas at 230 to 300 days of age (weight 433 to 664 gm.) the metabolism of the rapid growth rats was slightly lower than that of the slow growth rats (weight 307 to 402 gm.).

Male rats in the second and third years of life had a higher metabolism than females of the same age.

The basal metabolism of both male and female rats remained at practically a constant level during the second and third years of life. This finding is not in accord with an earlier observation on female rats from a different rat colony, that the heat production increased with increase in age between 2 and 28 months. Analysis of both sets of data indicates a very great difference in the body weights of the two groups of rats. The lack of agreement in the findings may be explained by the supposition that this difference in body size is not ruled out either in the expression of the heat production per unit of weight or that per unit of surface area.

## LITERATURE CITED

- BENEDICT, F. G. 1930 A multiple-chamber respiration apparatus for rats and other small animals. *J. Nutrition*, vol. 3, pp. 161-176.
- BENEDICT, F. G., AND M. F. HENDRY 1921 The energy requirements of girls from 12 to 17 years of age. *Boston Med. and Surg. J.*, vol. 184, pp. 217, 257, 282, 297 and 329.
- BENEDICT, F. G., K. HORST AND L. B. MENDEL 1932 The heat production of unusually large rats during prolonged fasting. *J. Nutrition*, vol. 5, pp. 581-597.
- BENEDICT, F. G., AND G. MACLEOD 1929 The heat production of the albino rat. II. Influence of environmental temperature, age, and sex; comparison with the basal metabolism of man. *J. Nutrition*, vol. 1, p. 381.
- BRUEN, C. 1933 Variation of basal metabolic rate per unit surface area with age. II. The pubertal acceleration. *J. Nutrition*, vol. 6, pp. 383-395.
- DONALDSON, H. H. 1924 The rat: data and reference tables. 2nd ed. *Memoir no. 6, The Wistar Institute of Anatomy and Biology*, Philadelphia, p. 177.
- DU BOIS, E. F. 1916 Clinical calorimetry. XII. The metabolism of boys 12 and 13 years old compared with the metabolism at other ages. *Arch. Intern. Med.*, vol. 17, pp. 887-901.
- HOGAN, A. G., AND R. PILCHER 1930 Growth and development with special references to domestic animals. XV. Energy and nitrogen metabolism during the first year of postnatal life. *Univ. Missouri, Agri. Expt. Sta., Research Bull.*, no. 143, p. 175.
- HORST, K., L. B. MENDEL AND F. G. BENEDICT 1930 The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. *J. Nutrition*, vol. 3, pp. 177-200.
- 1934 a The influence of previous exercise upon the metabolism, the rectal temperature, and the body composition of the rat. *J. Nutrition*, vol. 7, pp. 251-275.
- 1934 b The effects of some external factors upon the metabolism of the rat. *J. Nutrition*, vol. 7, pp. 277-303.
- OSBORNE, T. B., AND L. B. MENDEL 1915 Protein minima for maintenance. *J. Biol. Chem.*, vol. 22, pp. 241-258.
- 1919 The nutritive value of the wheat kernel and its milling products. *J. Biol. Chem.*, vol. 37, pp. 557-601.
- 1926 The relation of the rate of growth to diet. I. *J. Biol. Chem.*, vol. 69, pp. 661-673 (diet I on p. 671).
- OSBORNE, T. B., L. B. MENDEL, E. A. PARK AND M. C. WINTERITZ 1927 Physiological effects of diets unusually rich in protein or inorganic salts. *J. Biol. Chem.*, vol. 71, pp. 317-350.
- SHERMAN, H. C., AND H. L. CAMPBELL 1928 The influence of food upon longevity. *Proc. Nat. Acad. Sci.*, vol. 14, pp. 852-855.
- 1930 Further experiments on the influence of food upon longevity. *J. Nutrition*, vol. 2, pp. 415-417.
- SLONAKER, J. R., AND T. A. CARD 1923 Effect of a restricted diet. II. On pubescence and the menopause. *Am. J. Physiol.*, vol. 64, pp. 35-43.

- TOPPER, A., AND H. MULIER 1932 Basal metabolism of normal children: The puberty reaction. *Am. J. Dis. Child.*, vol. 43, pp. 327-336.
- WANG, C. C., J. E. HAWKS, B. HUDDLESTON, A. A. WOOD AND E. A. SMITH 1930 The influence of high and low protein diet on the basal metabolism and the chemistry of blood and urine in normal women. *J. Nutrition*, vol. 3, pp. 79-98.
- WINTERS, J. C., A. H. SMITH AND L. B. MENDEL 1927 The effects of dietary deficiencies on the growth of certain body systems and organs. *Am. J. Physiol.*, vol. 80, pp. 576-593.

# STUDIES ON THE EFFECT OF HIGH DOSES OF IRRADIATED AND NON-IRRADIATED ERGOS- TEROL ON THE ALBINO RAT <sup>1</sup>

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The very earliest studies with irradiated products indicated that they had a toxic action, and likewise the exposure of the animal to light also indicated toxic action. However, later studies led to irregular reports by various investigators. These irregularities were in part due to the fact that some of the investigators reported their dosages in terms of milligrams of ergosterol and not in terms of the therapeutic dose and also because the methods of irradiation used at that time undoubtedly introduced variable potencies and toxicity.

It is not our purpose to discuss all the papers on this subject, but to emphasize only the most important studies on rats. Thus the work of Rosenheim and Webster ('27) showed that 10,000 times the minimum antirachitic dose was not lethal to rats, while the work of Pfannenstiel ('28) and of Kreitmar and Moll ('28), using 'enormous doses,' claimed pathological findings for several species and also a species variation. Dixon and Hoyle ('28) fed a moderate excess of irradiated ergosterol to rats on a normal bread and milk diet and found no appreciable effect. Similar rats on a synthetic basal diet fed five and eight times larger doses failed to gain weight and on autopsy showed calcium phosphate in the urinary

<sup>1</sup> This work was undertaken on the suggestion of Mead Johnson & Co. who supplied the funds for the first year and who throughout the entire period have supplied us with the ergosterol preparations.

tract. Subsequently ('29) they fed even larger doses and substantiated the above findings. About the same time, Harris and Moore ('28) reported that on a synthetic diet, a dose 1000 times the minimum antirachitic dose was harmless to rats, while 100,000 times overdosage was rapidly fatal. The material used was ergosterol irradiated in an oily medium. They interpret their results to indicate that the irradiated ergosterol itself and not unchanged ergosterol or decomposition products was the factor responsible for the toxic action. These workers claimed to have obtained all the pathological findings reported by the earlier workers. Hoyle ('30), with rats on both synthetic and ordinary bread and milk diets, showed that when a synthetic diet was used he could readily obtain all the striking results of the earlier workers and also confirmed the findings of Harris and Moore. He further showed that if he fed high enough concentrations of irradiated ergosterol on a bread and milk diet he then could also obtain some toxic results although they were not as striking as on a synthetic diet. However, he showed that neither bread alone nor milk alone could prevent this condition, but that the two together acted as a preventative, and therefore concluded that there are two factors which one has to consider in the production of this condition, namely: a) a synthetic diet and, b) an excess of irradiated ergosterol. And finally, he showed that the toxic action was due to a) an excess of irradiated ergosterol and, b) an unknown toxic substance which was much more readily produced when the ergosterol was irradiated in an alcohol solution, being only difficultly produced when the irradiation was carried on in oil. Holtz and Schreiber ('30), working on mice, rabbits and dogs, confirmed the contention of Hoyle and showed that the antirachitic factor and the toxic factor are two separate principles. Furthermore, they showed that the antirachitic activity does not parallel the toxic action in all preparations. Seel ('29) showed that in rabbits large doses of irradiated ergosterol caused a marked loss of weight, emaciation and other cachectic symptoms, and that animals which were rachitic were more

sensitive to larger doses than were normally fed animals. Vara-Lopez ('30) showed that large doses of Vigantol caused changes in the spleen, adrenals and entire reticulo-endothelial system which were peculiar to the Vigantol solution not being given by Vigantol tablets, cod liver oil, or by cholesterol in oil. Goebel ('32) demonstrated that preparations of irradiated ergosterol differing as to industrial source or duration of activation differ also in their toxic effect when fed in massive doses. Bills and Wirick ('30) report experiments running over a longer period of time than the previous work, and showed that 1000 times the therapeutic dose caused just perceptible effects, 4000 times the therapeutic dose was definitely injurious, and 40,000 times the therapeutic dose was strongly toxic. In their work they used both the McCollum 3143 rachitic diet and the stock diet which is used in that laboratory and which cannot be considered as a bread and milk diet, according to Hoyle and his co-workers.

Our work was started in December, 1928. It was our object to maintain throughout our experimental procedures as normal conditions as possible and to limit our observations to only one commercial product which was prepared and furnished by Mead Johnson & Co.

#### EXPERIMENTAL METHODS

##### *A. Method of assay employed*

1. *Line test.* The method of assay used was to place a group of five rats weighing about 45 to 50 gm. on McCollum's diet 3143 for 14 days, at the end of which period the material to be assayed was fed separately to each rat. Two methods were employed in this individual feeding. In the earlier work, the material to be tested was first mixed with a small quantity of the rachitic diet and was then fed to the rats in separate cages and each animal was left in the cage until it had completely eaten its ration. The cod liver oil and earlier stocks of special Acterol were assayed by this feeding method. In the later work, a known concentration of the material in



peanut oil was fed to the animals by means of a stomach tube. This latter procedure seemed to us to be a more accurate measure of the amount actually utilized by the animals. The rats were fed for 10 days on this experimental diet and the bones examined according to the usual line test technic ('22). A therapeutic dose was considered to produce a ++ heal in 10 days.

All the feeding tests involving assay were carried out in a darkened room from which all possible sources of ultra-violet had been eliminated. The animals, when not eating their special diet, were kept in large cages equipped with false bottoms. All cages were thoroughly washed with boiling water and then sterilized in an autoclave once a week. All feed and drinking cups were well washed with hot water daily.

2. *Blood calcium and phosphorus methods.* Blood calcium and phosphorus were determined on two rats of each group on the fourteenth day of the experiment and again just before the rats were killed on completion of the assay. The blood was drawn by heart puncture, which yielded from  $1\frac{1}{2}$  to 2 cc. of blood without seriously affecting the rats. Because of the small volume of blood available, it was necessary to modify the regular methods and to use whole blood instead of serum. For inorganic phosphorus, the Fisk-Subbarow method ('25) was modified so that the blood and reagents were used in one-fifth quantities. For calcium, a modification of the Roc-Kahn procedure ('26) for calcium was devised so that the equivalent of 0.5 cc. of whole blood was used for duplicate estimations. In both determinations, a microcolorimeter was used.

### *B. Routine procedures in the high dosage experiments*

1. The animals were fed either the regular stock diet or the rachitic diet plus a definite quantity of Superacterol per rat per day. This extra ration was fed in three different ways. In the earlier work, it was mixed with the regular stock diet, while in the later work, where it was desirable to use higher

doses, we first used a carefully calibrated medicine dropper which was eventually replaced by a graduated stomach tube.

2. All animals were weighed weekly and the growth curves plotted.

3. The behavior of the animals was observed and a careful record of any irregularities kept.

4. Records were kept of matings and of the weight, size and condition of all litters.

5. Animals sacrificed or found dead were carefully autopsied. The organs of the chest and abdomen were carefully examined grossly. The urinary tract was usually dissected out intact and examined under the dissecting microscope for possible calculi. The following organs were carefully weighed: thymus, heart, lungs, liver, spleen, kidneys, and, in the males, the testes.

6. For microscopic study sections were prepared from the thyroid, thymus, aorta, heart, lungs, liver, stomach, duodenum, spleen, kidneys and gonads.

#### EXPERIMENTS AND RESULTS

##### *A. Standardization of cod liver oil and Acterol*

Our assays showed that 0.3 cc. per rat per day of a solution composed of one part of cod liver oil and twenty-nine parts of peanut oil gave a good (++) cure in 10 days. Control experiments showed that the peanut oil used had no detectable activity. On the basis of the statement that the Acterol used was one hundred times as potent as the cod liver oil, we fed a solution of Acterol in which the Acterol was one one-hundredth the concentration of the cod liver oil fed above. This gave us a cure which was slightly better than ++, but which we believed to be within the limits of biological assay. In another assay on a still more potent solution, we used the stomach tube method and found that the strength of the solution was exactly what it was calculated to be. This latter material was a solution of Mead-Johnson's Viosterol which had a potency of 10,000 D  $\pm$  17 per cent. The studies on the cal-

cium and phosphorus showed that there is a more or less distinct relation between the blood and bone pictures. In severe rickets, on the fourteenth day of the rachitic diet, the calcium varied from 6.5 to 7.5 mg. per 100 cc. of whole blood, while the inorganic phosphorus values ran from 1.5 to 3.0 mg. per 100 cc. After being on a curative ration for 10 days following the 14 days on a rachitic diet, the values were found to range from 8.0 to 9.5 mg. per 100 cc. for calcium, and from 4.0 to 6.0 mg. per 100 cc. for phosphorus. When the test ration was not potent enough to give any cure, the values for calcium and phosphorus became even lower than they were on the fourteenth day of the rachitic diet.

### *B. High dosage experiments*

*Series 1. Feeding experiments with high dosages of irradiated ergosterol given to albino rats on a standard normal diet.*

(a) Eighteen normal albino rats, 5 weeks old, placed on the normal stock diet, were divided into four groups containing about the same number of males and females. Each one of these groups was fed, in addition to its normal diet, either a special dose of Acterol (1:100) or an equivalent amount of non-irradiated ergosterol in peanut oil. The Acterol or ergosterol was fed to each animal individually once each day. These additional diets were made up in the following way:

Group I received a diet calculated to contain one hundred times the therapeutic rat dose of Acterol. In all our calculations we adopted the therapeutic dosage as suggested by Mead Johnson & Co. According to their standards, about 1/80 gm. of cod liver oil per rat per day gives a good ++ line test in 5 days, while Acterol is one hundred times as potent. On this basis the diet of group I was made up by taking 100 cc. of Acterol and diluting it with 91 cc. of peanut oil. We estimated that one standardized drop of this mixture would be equivalent to one hundred times the therapeutic rat dose of Acterol.

Group III received an extra ration of four drops of undiluted Acterol (1:100) per rat per day, which is equivalent to approximately eight hundred times the therapeutic rat dose of Acterol.

Groups II and IV received diets containing unirradiated ergosterol in amounts equivalent to the irradiated ergosterol present in the Acterol of the diets of groups I and III, respectively. Based upon the statement of Mead Johnson & Co., that Acterol contains 50 mg. of ergosterol in 140 cc. of oil, the diet of group IV was 4 drops per day of a solution of 50 mg. unirradiated ergosterol completely dissolved in 140 cc. of peanut oil. Group II received as an extra ration 1 drop of a mixture of 100 cc. of the above solution of unirradiated ergosterol and 91 cc. of peanut oil.

This experiment was continued over a period of 42 weeks (3/4/29 to 1/3/30). The weights of the animals in these four groups showed that growth was fairly normal. No distinct gross pathology was observed. Several of the rats in group I showed pathological changes in the lungs, but this was diagnosed as pneumonia, a very common rat ailment, and could, therefore, not be attributed to the Acterol fed. All the females in group I and one female in group III failed to reproduce which at first led us to believe that a factor of sterility might be involved. This experiment has been repeated many times with animals receiving ten and a hundred times as much of the same lot of Acterol without producing any similar conditions, and we therefore conclude that the failure in reproduction was due to some factor other than Acterol.

(b) The second experiment under this general heading was started with ten 6-month old rats. These animals were fed a normal diet with the exception that the animals in group V received an extra individual ration consisting of ten thousand times the therapeutic rat dose of Acterol, while the four rats of group VI were fed an extra daily ration of unirradiated ergosterol in peanut oil equivalent to the concentration of ergosterol in the diet of the rats in group V. The actual

amount of extra material fed in this case was 7 drops of Superacterol which is six times as potent as Acterol, according to Mead Johnson & Co. assay. This experiment was carried on for a period of 3 years and 15 weeks (4/5/29 to 7/20/32). As the first and second generation animals were obtained they were immediately, on weaning, placed on the same test diet as their parents. In some of the later work the young were fed the test diet directly by means of a medicine dropper beginning at the first or second week after birth.

In summing up the work on these groups, we can say that there was very little if any difference in the growth of the two groups or their offspring. Several of the young in group V died shortly after weaning, but this may be explained by the fact that in these cases the mother had been mated too young and the whole litter was not up to the standard. Furthermore, the later work, when greater care was taken in mating, shows no such picture. Throughout this work no gross or microscopic pathology was observed except that which was due to pneumonia. Even those young which received this large dose of Superacterol when only a week old appeared perfectly normal in all respects upon weaning. Four of these young animals, of the fourth generation, were selected and placed on an extra ration of 15 drops of a new lot of Superacterol which had a potency of 1000 D. These animals received approximately 36,000 times the therapeutic rat dose over a period of 2 years and 21 weeks (2/21/30 to 7/20/32), and seemed to be normal in all respects. The three females all gave birth to normal litters. One of the females was killed during the thirty-first week of the experiment and a careful study of her tissues showed no pathology. During the forty-second week of this experiment the three remaining animals were placed on 20 drops of Superacterol which was equivalent to approximately 50,000 times the therapeutic rat dose. The male in this group was killed in the fiftieth week of the experiment and again the tissues were studied for evidences of pathological lesions with no results. The second female of this series died during the sixty-sixth week of the

experiment and again there were no signs of gross or microscopic pathology. The last animal, female 153, died in the sixty-ninth week of the experiment, while blood was being drawn from the heart by means of heart puncture.

Eighteen of the fifth-generation animals from the litters of the three fourth-generation females were observed over a period of 43 weeks (10/9/30 to 8/25/31), while receiving approximately 36,000 times the therapeutic rat dose and at no time did they exhibit any pathological symptoms. Twelve young animals of the sixth generation were observed under the same conditions over a period of 18 weeks (4/25/31 to 8/20/31) without showing any toxic symptoms. This is the shortest period of observation for any of the animals in this series. The total number of animals in this experiment was 127 and they were divided in the following manner: 10 first-generation adults, 48 second, 35 third, 4 fourth, 18 fifth, and 12 sixth-generation young animals.

(c) The third experiment under this general heading was started with five normal animals 4 weeks of age. These animals were fed a normal stock diet which was made up so that it contained 25 mg. of irradiated ergosterol per 10 gm. of original diet. This ergosterol was irradiated in the dry state by a Cooper-Hewitt mercury vapor lamp; the time of irradiation was 35 minutes at a distance of 40 cm. An accurate record of the food consumption of each rat was kept and it was found that on an average each rat ate 10+ gm. of food per day which meant that each animal was getting 25+ mg. of irradiated ergosterol per day. An assay of this irradiated ergosterol showed that it gave a good ++ cure in 10 days when fed in doses of 0.001 mg. per rat per day. Thus the animals in this experiment were receiving 25,000 times the therapeutic rat dose. These animals were kept under observation for 23 weeks (8/12/29 to 1/24/30), and showed absolutely no evidences of hypervitaminosis. The offspring of these animals were also put on the same diet and observed for 10 weeks (1/13/30 to 3/28/30), and likewise gave no signs of abnormality.

*Series 2. Feeding experiments with high doses of irradiated ergosterol given to albino rats on a rachitic diet.* (a) The following experiment was designed to study the possible toxic effects of excessive doses of Acterol on animals which were on an otherwise rachitic diet. Sixteen rats, 4 weeks of age, were placed on the McCollum diet 3143 for a period of 14 days. At the end of that time ten of them were fed in addition a quantity of Acterol equivalent to ten thousand times the therapeutic dose. The remaining six rats were fed on a control diet of unirradiated ergosterol in peanut oil. With the exception of female 22 all the animals on Acterol showed a fairly normal growth for the first 5 weeks, although the rate of gain was slightly lower than normal. Female 22, the exception to the above, commenced to lose weight as soon as she was put on the curative diet and finally in the fifth week on curative diet she was killed and an autopsy performed, the report of which is herewith given:

Although this animals had lost 10 gm. in weight (80 to 70 gm.) during the 31 days on the test diet, at autopsy no definite pathology was identified. No calcium deposits were noted in any of the tissues nor were there any proliferative changes or necrosis such as have been described by other workers as preliminary to the pathological calcification which they have reported. No calculi were present in the urinary tract. The great vessels were elastic and were not sclerosed or calcified.

After the fifth week the remaining animals in this group began to show distinct fluctuations in growth. This was true in all cases except that of female 34 which showed a constant though slow gain. Male 28, although he had been gaining weight slowly, suddenly developed a distaste for his food and refused to eat. He next developed a marked diarrhea and an enormous distention of the bladder and finally, having eaten very little for 2 or 3 days, he went into distinct tetanic convulsions in which he died. The autopsy showed these conditions:

This animal had a bladder which measured 20 x 20 x 25 mm. Both ureters were distended and the kidneys were enlarged.

No calculi were found. The kidneys weighed together 2.33 gm., about double the normal weight for this size animal. The spleen seemed abnormally small. The microscopic picture was that of a hydronephrosis. No line of calcification at the junction of cortex and medulla was seen as reported by Dixon and Hoyle ('28). All other tissues were normal.

In order to make a comparative pathological study, female 132 of this group was sacrificed as was also a control female of the same age. Studies by various different staining techniques for showing calcium, which will be described in a subsequent paper on the microscopic findings, showed no differences between the two animals which could be classified as pathological.

Male 23 showed a distinctly poor growth curve although his appetite was good. His total gain in weight at the peak of his growth curve in the twenty-third week, was only 49 gm. Besides this he showed a distinct prolapse of the penis. The penis, hanging out from the body, had the appearance of a loose bluish-colored appendage of the skin. The other male in this group, 35, though underweight, seemed to be normal in all respects.

The three animals in this control group on unirradiated ergosterol showed no great differences from the above group. Female 36 which showed an irregular growth curve died during the twenty-second week and the following gross changes were observed:

There was some thinning of the hair especially over the back, moderate emaciation and considerable paleness of the tissues except the lung, which was bright red. The liver showed the lobular markings quite prominently and an area at one margin 5 x 3 x 2 mm. in which the liver tissue appeared pale yellowish and caseous. This was surrounded by a dark hemorrhagic zone about 1 to 2 mm. wide. All other tissues examined appeared normal although no thymus tissue could be identified.

Of the two males in this control group, male 38 exhibited the symptoms listed above of flaccid penis, etc., and male 42



seemed quite normal in all respects except in weight. However, this male showed a better and more consistent gain in weight than any animal in either group.

In reviewing the work on this group which was in progress for 51 weeks (2/27/29 to 2/21/30), we find that all the animals in the control group died except male 42, which seemed to be perfectly normal in all respects. In the group receiving the Acterol all animals except female 34 and male 35 died. These two animals appeared almost normal although they were not quite as sleek looking as our stock animals. Female 34 had one litter of five out of which three died before they were weaned. It is our impression that the irregularities in growth and development in the animals in this group were due to the deficiency of the diet rather than to any effects of the ergosterol feeding.

The most striking observation, however, was the fact that none of the animals, in the control group, which came to autopsy, showed active rickets although they had been on a vitamin D free diet. There are several reasons which might have been the cause of this situation. In the first place, three of the animals in this group died and were eaten by their litter mates before we were aware of their death. In this way the phosphorus and the small amount of residual vitamin D in the tissue of the dead animals was made available to the survivors. Next, those which were autopsied showed a slight loss of weight before death or else had not shown any gain for several weeks. It is known that in order to show a typical rachitic bone picture at autopsy an animal must have shown consistent growth throughout the experimental period. Another factor which may have been of some importance was the fact that after the twenty-third week of the experiment, the animals were not kept in a completely darkened room, although they were never exposed to the direct sunlight. Then, finally, in feeding such a high concentration of ergosterol it might be possible that there was a sufficient contamination by active material to cause the animals to show a normal bone picture.

*Series 3. Feeding experiments with extremely high doses of irradiated ergosterol given to albino rats on a normal diet, with and without potassium iodide, and on a rachitic diet without iodide.* (a) The negative results which were obtained in the above experiments led us to start an experiment in which extremely large doses were given to the animals in an effort to see whether we could obtain any of the toxic symptoms reported by others. In this series, we used the Mead-Johnson Viosterol which had a potency of 10,000 D. All test materials were fed by stomach tube. We started with fifty-two albino rats which were divided in the following manner:

Group VII. Twenty-seven animals received the normal stock diet plus 93,000 times the dosage of Viosterol.

Group VIII. Twenty-five animals received as an added daily ration 93,000 times the therapeutic rat dose of Viosterol and also various amounts of potassium iodide. The iodide was added in this case because of the claims made by Simonnet and Tanret ('30), who maintained that the addition of large quantities of iodide prevent the calcification due to excessive doses of irradiated ergosterol.

Group IX. Twenty controls, ten of which received a quantity of peanut oil equivalent to the amount received by the experimental animals, and the other ten received an amount of potassium iodide equivalent to the amount fed to the test animals.

This experiment was continued over a period of 37 weeks (11/6/31 to 7/21/32). As far as the gross condition of the rats is concerned, the feeding of the iodide had neither a deleterious nor advantageous effect. The animals seemed normal in all respects and no gross pathology was observed.

It was, however, felt desirable to carry this experiment on a little further, so three of the healthiest males of group VII were taken and fed an amount of Viosterol equivalent to the 465,000 times dosage. Some very striking results were now obtained. Two of these animals died on the fifth day of feeding and the third showed a distinct loss in weight, a

general emaciation, a slight diarrhea and a definite loss of appetite with an associated general apathy. Two of the animals showed a distinctly bloody urine and the last animal to die showed very marked evidences of calcification in the aorta. This striking finding led to a continuation of the experiment and this was done by starting nine young animals on this high dosage. In this case three animals died during the first week, four during the second week, and the rest during the third week.

(b) In order to satisfy ourselves as to the validity of these results we next treated twenty-seven normal adults as follows:

Group X. Thirteen normal females, 9 months old, who had all had one normal litter, were so divided that nine of them received the 465,000 times dosage of Viosterol, while the remaining four received the 93,000 times dosage.

Group XI. Seven normal females, and seven normal males, all 3 months of age, were so divided that five females and six males received the 465,000 times dosage, while the balance of two females and one male received the 93,000 times dosage of Viosterol.

All animals on the 465,000 times dosage died within 5 weeks; 11 females and 2 males in the first, 2 females and 3 males in the second, 1 male in the fourth and 1 female in the fifth week. All developed a slight diarrhea, loss of weight, a flabby emaciated musculature, inactivity and loss of appetite. Most of them showed a bloody nasal discharge which appeared to be associated with a difficulty in breathing. All of the rats on the 93,000 times dosage survived during this period although some of them did not appear to be normal and had lost considerable weight. The blood from several of these animals was analyzed for calcium and inorganic phosphate, and no significant rise was noted, which is in agreement with the report of Jones, Rapoport and Hodes ('30).

(c) A third experiment under this general heading was now started and this involved the use of eighty-four normal animals of approximately 4 weeks of age. These animals were divided into three major groups each of which was again

subdivided into three smaller groups according to the following scheme:

Group XII. Thirty animals were placed on a rachitic diet for a period of 2 weeks, at the end of which time they were kept on the same basic diet and received in addition 46,500, 93,000 and 465,000 times the therapeutic rat dose of Viosterol, respectively.

Group XIII. Twenty-seven rats were placed on a rachitic diet for a period of 14 days at the end of which time they were placed on a normal diet and divided into the three sub-groups listed above.

Group XIV. Twenty-seven animals were placed on a normal stock diet for a period of 2 weeks, at the end of which time they were divided into the three sub-groups referred to above, but continued on the normal basal stock diet.

In this experiment, we wished to determine three things, 1) were the animals more resistant to this toxicity when on a McCollum vitamin D free diet than when on a normal diet; 2) what was their reaction when rachitic and then replaced on a normal diet plus the overdosage of irradiated ergosterol; and, 3) the difference between the various levels fed under the different conditions of the experiment. The results obtained under these conditions were quite clear cut and definite. All of the animals receiving the 465,000 times dosage with McCollum's vitamin D free diet were dead by the sixth day following the first dose of irradiated ergosterol. These animals all showed evidences of emaciation with an extensive loss of weight, all exhibited signs of diarrhea and all had a bloody discharge from the nose. At autopsy no gross pathology of the organs was observed. The animals receiving the 93,000 times dosage did not show the extreme symptoms listed above, but they did show a steady loss of weight and by the end of the third week were so weak that they could hardly move about. All of these animals died or were killed during the fourth week. Some of these animals had a slight diarrhea and all had a more or less bloody nose. The main gross autopsy findings were that in all but two of the animals the

liver and kidneys were a pale grayish brown color speckled with darker brown dots. In female 500 the lung was full of caseous nodules while in male 497 the spleen was very friable. The animals receiving the 46,500 times dosage appeared normal in growth and appearance for the first 2 weeks, and then all but four of them also began to lose weight, although their loss was only gradual. These animals were all killed during the fifth week of the experiment and we were unable to observe any gross pathology other than the loss of weight.

In the group of animals which was transferred from the rachitic to the normal diet at the end of the second week, several characteristic findings were observed. Of the animals receiving the 465,000 times dosage, two died during the first week, seven during the second week and the remaining animal died during the third week of the experiment. Here again the animals had lost much weight and at autopsy we observed that the kidneys were of a very pale grayish brown color, speckled with darker brown dots, and on cutting through the kidneys it was seen that this was the color of the entire cortex. The thymus in all these animals was quite small and the adrenals appeared larger than those of normal animals. The hearts of these animals showed a yellowish mottling against the background of the darker myocardium. The animals which were receiving the 93,000 times dosage appeared quite normal during the first week of the experiment and with the exception of two animals which lost slightly in weight, appeared quite normal during the second week. All of these rats were killed during the third week of the experiment and no gross evidences of pathology were observed. Those animals receiving the 46,000 times dosage appeared perfectly normal and when killed during the third week showed absolutely no symptoms of any gross pathology.

The groups receiving a normal diet from the time of weaning, and which were 2 weeks later placed on the 46,500 and 93,000 times dosage were normal in all respects and at autopsy during the third week gave no evidences of any gross pathol-

ogy. The animals receiving the 465,000 times dosage again showed all the symptoms listed in the previous two groups on this level of Viosterol, namely, pale speckled kidneys, mottled heart, small thymus and large adrenals. All but one of these animals were dead by the end of the second week of the experiment and this animal was killed during the third week. The arch of the aorta of this animal (male 564) showed signs of calcification. In order to assure ourselves that we were observing all the gross pathology, we killed two groups of three animals each, consisting of litter mates on the three levels of irradiated ergosterol fed. The tissues of these animals were then compared grossly with each other and with the tissues of a normal untreated animal of the same age. When this was done it was seen that the thymus of the animals on the 465,000 times dosage was less than one-fourth the weight of the thymus in the other treated animals and in the normal control. In fact, in the subsequent animals brought to autopsy this relationship was in most cases much more pronounced. This comparative study also emphasized the previously noted difference in the size of the adrenals and also definitely brought out the fact that the myocardium of the highest dosage animals was affected in some manner by the production of slightly elevated areas of a yellowish mottling which did not appear to correspond to the major vessels of the myocardium. Furthermore, this study substantiated our belief that, at least from a consideration of gross pathology, the animals receiving the 46,500 and the 93,000 times dosage were in all respects normal. Finally, in order to determine whether the change in the thymus was an atrophy or merely a cessation of growth, several normal animals, of the age of the experimental ones at the time when they were first fed ergosterol, were killed and it was observed that in all these cases the thymus of the untreated animals was two to three times larger than the thymus of the highest dosage animals and that in all instances the thymus was smaller than the thymus of the animals receiving the 46,500 and 93,000 times dosage, respectively, or than the thymus of the older normal controls.

*Series 4. Experiments on the protection of the young against rickets.* With so many young animals available from the other experiments in progress, we thought it would be interesting to check the findings of Bills and Wirick ('30) as to the influence of activated ergosterol in the mother's diet on the resistance of the young to rickets. Seventy-six animals were used in this work. These animals were from mothers receiving 10,000 times the therapeutic rat dose of irradiated ergosterol and were placed on McCollum's rachitic diet 3143 at weaning. At the end of a 3-week period the animals were killed and studied by the line test and blood analyses for rickets. In all cases, there was only a slight degree of rickets.

Another experiment involving thirty-seven rats was run. In this experiment the mothers were receiving an extra ration of 10,000 times the therapeutic rat dose of irradiated ergosterol and immediately after birth the young were given to a foster mother on normal diet. All of these animals showed the characteristic rachitic blood analyses and line test after having been on the rachitic diet for 3 weeks. These results definitely confirm the findings of Bills and his co-workers ('30).

#### DISCUSSION

From the data presented it seems clear that rats when fed a normal bread-milk diet are not affected by irradiated ergosterol if given 46,500 times the therapeutic rat dose. When similar animals are given 93,000 times the therapeutic rat dose of irradiated ergosterol some toxic symptoms are observed. The effect of this dosage is, however, quite different on young and adult animals. When young animals are given this amount of irradiated ergosterol, they show no immediate symptoms; in fact, they develop quite normally and it is only as they approach the age of 9 to 12 months that they begin to manifest any symptoms of toxicity and these are not extremely serious, having in no case in our series led to the death of the animal. When 93,000 times the therapeutic rat dose of irradiated ergosterol is fed to animals 3 and 9 months old, respectively, there is an immediate loss

in weight which is much more pronounced in the older animals. In some cases this dosage led to death of the animals with the same gross pathological findings as are observed in animals on a higher dosage. It is quite significant to note that there is a definite difference in the reaction of different adult animals when on this level of irradiated ergosterol. This is very strikingly brought out in the seven rats on this dosage in groups X and XI; of these, two rats, 675 and 619, died while the remaining animals appeared quite normal except for slight loss in weight. This experiment also shows the difference between the reaction due to age, for the animals which were 3 months of age lost very little weight and none of them died while the 9-month-old animals lost weight steadily and two of them died.

When 465,000 times the therapeutic rat dose of irradiated ergosterol was fed to either young or adult animals there was an almost immediate toxic response. This reaction was characterized by the following symptoms: immediate loss of weight, the appearance of diarrhea and of a bloody discharge from the nose, the development of a muscular flabbiness, a definite loss in appetite and a very marked emaciation. When brought to autopsy these animals showed definite gross pathology. There were varying degrees of calcification of the aorta, the kidneys were a very pale grayish color and speckled with darker brown dots, the thymus was atrophied, the adrenals were larger than in the normal, and the myocardium showed a grayish yellow mottling. Here again we can see the greater susceptibility of the adult animals to these high dosages. Seventeen of the adult animals fed on 465,000 times the therapeutic rat dose of irradiated ergosterol and a normal diet were dead by the end of the fifth day, one lived for 2 weeks, while the last two rats lived for 3 weeks. Of the nineteen young animals on this level of irradiated ergosterol and a normal diet only three were dead at the end of the first week while the other sixteen died or were killed between the thirteenth and seventeenth days of the experiment.



Certain workers (Kreitmair and Hintzelmann, '28; Krietmair and Mell, '28, and Pfannenstiel, '27, '28) have also reported the atrophy of the spleen to be one of the results of excessive doses of irradiated ergosterol, but we could not confirm this finding. The weights of the spleens in our animals varied considerably and at times we thought we were confirming this finding. However, when we selected the records of all the animals receiving irradiated ergosterol for which there was a control rat of approximately the same weight, we found that the total weight of the spleens of the animals receiving irradiated ergosterol was 0.37 per cent of the total gross weight, while the total weight of the spleens of the control animals was 0.38 per cent of the total gross weight. From these facts we feel justified in saying that we have not the slightest evidence of any splenic atrophy in our rats. Perhaps the most consistent and outstanding pathology we observed was in the thymus. In the eighty-four animals in groups XII, XIII and XIV, the sixty-five animals on the 46,500 and 93,000 times dosages of irradiated ergosterol as well as four normal controls, had a thymus anywhere from three to four or more times as heavy as any of the animals on the 465,000 times dosage. In fact, in many cases the thymus of the highest dosage animals was so small as to be almost unrecognizable and unweighable.

Many investigators have reported the finding of calculi in the urinary tract which have given rise to hydronephrosis. In our series, we had one rat receiving 10,000 times the therapeutic dose of irradiated ergosterol and McCollum's rachitic diet 3143 which developed hydronephrosis due to an obstruction in the bladder or lower down. We were, however, unable to locate a calculus although it may have been present. Another rat had a mild distention of the bladder and a small calculus 1 mm. in diameter was found at the urinary trigone. However, the largest calculi we found were in a control animal on a rachitic diet. There were two of them, each of which measured  $3 \times 3 \times 6$  mm. They were crystalline in structure and gave a strong test for calcium, but only a very faint phosphate test.

Brown and Shohl ('30) claim that animals on a rachitic diet and receiving large overdoses of irradiated ergosterol (Vigantol) are more resistant to the toxic effects than animals on a normal diet. We cannot subscribe to this claim, for in our work we found that the animals on a rachitic diet showed the toxic effects much more rapidly and at a lower level than those on a normal diet. Of the animals in group XII, the ten animals receiving McCollum's rachitic diet 3143 and 465,000 times the therapeutic rat dose of irradiated ergosterol were all dead by the end of the first week, and the ten animals receiving the same diet and 93,000 times the therapeutic rat dose of irradiated ergosterol either had died or were killed by the end of the fourth week because of their extremely weak condition. In contrast to this only three of the animals in groups XIII and XIV receiving the 465,000 times dosage died during the first week, while all but two of the remainder died or were killed during the second week and the last two during the third week. Furthermore, of the eighteen animals in these groups receiving the 93,000 times dosage, only two animals showed a very slight loss of weight at the end of the second week and these two animals were from group XIII and had been on a rachitic diet during the first 2 weeks of the experiment. The other animals were normal in all respects. The difference in these findings may be due to the fact that the rachitic diet used in our work was a much more severe diet than that used by Brown and Shohl. However, in their first paper ('30) they compare animals which had been on a rachitic diet plus irradiated ergosterol for 6 days with animals which had been on a normal diet plus the same amount of irradiated ergosterol for 11 days and we feel that such a comparison is not warranted. In their second paper ('30) these workers do not present enough clear cut experimental evidence to uphold their contention that on a rachitic diet the toxic effects are not as pronounced as on a normal diet.

A detailed discussion of the gross and histological specimens of all the experimental animals will appear in a later publication.

## SUMMARY AND CONCLUSIONS

1. Commercial preparations of irradiated ergosterol of exceptional potency were standardized by the McCollum line test and found to possess the claimed activity.

2. Ten albino rats were placed on a stock diet plus 100 to 800 times the therapeutic rat dose of this preparation for 36 weeks without observing harmful effects.

3. The progeny of 48 second, 35 third, 4 fourth, 18 fifth and 12 sixth generation rats were placed on the same stock diet plus doses up to 50,000 times the curative dose without observing harmful effects. The sixth generation animals were treated for 18 weeks, all others were treated for longer periods.

4. Pure ergosterol was irradiated in the solid form by a Cooper-Hewitt mercury arc lamp and fed to albino rats and their offspring in amounts up to 25,000 times the curative dose for 10 to 23 weeks without harmful effects.

5. Albino rats were placed on the McCollum 3143 diet with and without the commercial preparation in amounts up to 10,000 times the therapeutic rat dose. Although most of the animals died in less than 30 weeks, no true hypervitaminosis could be detected.

6. Young and adult albino rats were kept on a stock diet including bread and milk, plus 46,500 times the therapeutic rat dose for 3 weeks without harmful effects.

7. Young albino rats were placed on a stock diet including bread and milk plus 93,000 times the curative dose for 12 months when some of them showed loss of weight. Adult animals under the same conditions showed immediate loss of weight and in some cases died with pathological findings similar to those indicated below.

8. Young and adult albino rats were placed on a stock diet including bread and milk plus 465,000 times the therapeutic rat dose with the appearance of immediate toxic effects. There were immediate loss of appetite and weight, bloody discharge from the nose, diarrhea, marked muscular flabbiness, and gross pathological changes involving enlarged supra-

renals, atrophied thymus and abnormal appearance of the kidney and heart. The adult animals were again more susceptible than the young ones.

9. Rachitic diet was substituted for the stock diet on the 46,500, 93,000 and 465,000 dosage experiments on young animals. This change made the animals more susceptible.

10. Potassium iodide was added on the dosages of 93,000 times the curative amount without changing the picture.

11. Young from mothers on 10,000 times the therapeutic rat dose were weaned and placed on the rachitic diet. Only very slight rickets developed. Similar young, but nursed by stock diet foster mothers, developed typical rickets.

12. These results indicate that the toxic effects observed by others on relatively low dosages of irradiated ergosterol are due to the presence of a toxic substance other than the true antirachitic agent in appreciable amounts in the preparations tested. Whether the toxic effects observed by us on our commercial preparation when given in the very high doses are due to a trace of this hypothetical by-product or to the vitamin D substitute remains to be determined.

13. Our results again indicate that the toxicity of a given irradiated ergosterol preparation is also determined in part by the character of the diet. In general, the more complete and better balanced diets act in a more protective manner.

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#### LITERATURE CITED

- BILLS, C. E., AND A. M. WIRICK 1930 Long time feeding experiments with activated ergosterol. I. *J. Biol. Chem.*, vol. 86, p. 117.
- BROWN, H. B., AND A. T. SHOHL 1930 Rickets in rats; alterations of calcium and phosphorus metabolism of normal and ricketic rats produced by irradiated ergosterol. *J. Biol. Chem.*, vol. 86, p. 245.

- DIXON, W. B., AND J. C. HOYLE 1928 The effects of irradiated ergosterol in large doses. *Brit. Med. J.*, vol. 2, p. 832.
- FISKE, C. H., AND Y. SUBBAROW 1925 A micro method for phosphorus. *J. Biol. Chem.*, vol. 66, p. 375.
- GOEBEL, F. 1932 Sur la toxicité de la vitastérine D. *J. de Physiol. path. gén.*, T. 30, p. 379.
- HARRIS, L. J., AND T. MOORE 1928 Hypervitaminosis and vitamin balance. *Biochem. J.*, vol. 22, p. 1461.
- HOLTZ, F., AND E. SCHREIBER 1930 Einige weitere physiologische Erfahrungen über das bestrahlte Ergosterin und seine Umwandlungsprodukte. *Zeit. physiol. Chem.*, Bd. 1, S. 191.
- HOYLE, J. C. 1930 Further observations on toxic effects of irradiated ergosterol. *J. Pharm. and Exper. Therap.*, vol. 40, p. 351.
- HOYLE, J. C., AND H. BUCKLAND 1929 Further observations on the effects of large doses of irradiated ergosterol. *Biochem. J.*, vol. 23, p. 358.
- JONES, J. H., M. RAPOPORT, AND H. I. HODES 1930 Sources of excess calcium in hypercalcemia induced by irradiated ergosterol. *J. Biol. Chem.*, vol. 89, p. 647.
- KREITMAIR, H., AND W. HINTZELMANN 1928 Über sklerotische Organveränderungen, insolderheit der Arterien. *Arch. exp. Path. und Pharmacol.*, Bd. 137, S. 203.
- KREITMAIR, H., AND W. MOLL 1928 Hypervitaminose durch grosse Dosen Vitamin D. *Münch. Med. Woch.*, Bd. 15, S. 637.
- MCCOLLUM, E. V., N. SIMMONDS, P. G. SHIPLEY, AND E. A. PARK 1922 Studies on experimental rickets. *J. Biol. Chem.*, vol. 51, p. 41.
- PFANNENSTIEL, W. 1927 Med.—naturw. Gesellschaft zu Munstr., *Klin. Woch.*, Bd. 48, S. 2310.
- 1928 Weitere Beobachtungen über Wirkungen bestrahlten Ergosterins in Tierversuch. *Münch. med. Woch.*, Bd. 26, S. 1113.
- ROE, J. H., AND B. S. KAHN 1926 The colorimetric determination of blood calcium. *J. Biol. Chem.*, vol. 67, p. 585.
- ROSENHEIM, O., AND T. A. WEBSTER 1927 Parent substance of vitamin D. *Biochem. J.*, vol. 21, p. 389.
- SEEL, H. 1929 Wirkungen und Nebenwirkungen des bestrahlten Ergosterins. *Münch. med. Woch.*, Bd. 76, S. 1413.
- SIMONNET, H., AND G. TANRET 1930 Sur la toxicité pour les animaux de laboratoire des hautes doses d'ergostérol irradié. *Bull. soc. chim. biol.*, T. 12, p. 371.
- SHOHL, A. T., H. GOLDBLATT, AND H. B. BROWN 1930 Pathological effects upon rats of excess irradiated ergosterol. *J. Clin. Invest.*, vol. 8, p. 505.
- VARA-LOPEZ, R. 1930 Über den Einfluss des Vigantols auf den Tierkörper. *Klin. Woch.*, Bd. 9, S. 1072.

# FACTORS WHICH DETERMINE RENAL WEIGHT

## XVI. THE NATURE OF THE PROTEIN INTAKE

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### ONE FIGURE

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The protein intake has been found to be (Mac Kay, Mac Kay and Addis, '28 a, '28 b; Mac Kay and Mac Kay, '31 a, '31 b) one of the most important of the factors which determine the weight of the kidneys in the albino rat. The relationship between the protein intake and renal weight when casein is the protein, may be expressed for male rats of all ages by the empirical formula,

$$\text{renal weight} = (\text{protein intake} + 2.75) \div 0.0183$$

In the study reported here we were interested in whether or not this relationship was limited to casein or held generally for natural protein products.

Four sources of protein, namely, crude commercial casein, commercial 'egg albumin,' commercial 'blood albumin' and gelatin were used. Except for gelatin these were fed separately. For reasons detailed later gelatin was fed in combination with casein. The method of experiment was identical with that used before (Mac Kay and Mac Kay, '31 a). A group of about twenty male albino rats were fed upon each diet from 26 to 70 days of age. The diets which were used are described in table 1.

Growth on the various diets was interesting. In table 2 are depicted the percentage changes in body weight for each



group. The increase in body weight was greatest on the casein diets. The 'blood albumin' diets also gave good growth. In both cases the 46 per cent protein diets were optimal. Both the egg albumin (with the exception of the diet with the lowest concentration) and the gelatin diets gave poor growth and the higher the concentration of these proteins the worse the increase in body weight. The significance of the weight changes is borne out by a comparison of the average body lengths (table 3) at death.

Numerous rats receiving the egg white diets developed the 'pink disease' described by Boas ('27) and Findlay and Stern ('29) as occurring when raw egg white (as our product was) is fed to rats. Except for the loss of hair and the thickening and redness of the skin there were no other symptoms and the animals appeared to be in fairly good condition. The more serious symptoms noted by Parsons ('31) were not observed. The affected rats were distributed throughout all three diet groups so that the occurrence of this 'egg white disease' in its mild form was probably without influence upon the results.

We have noted the incorporation of casein in the gelatin diets. Some years ago an attempt was made to feed rats on diets containing gelatin as the sole source of protein. A commercial<sup>1</sup> powdered gelatin was used. Growth was poor but the animals were really in very good shape until they suddenly died in uremia. Further examination disclosed a marked proteinuria before this time and at autopsy the kidneys were twice their normal size and weight. They were firm and gray in color. Histological examination disclosed extreme epithelial degeneration. Later on these findings followed the feeding of a purer gelatin.<sup>2</sup> These brittle sheets were difficult to pulverize and casein was incorporated in one diet to overcome this mechanical difficulty. This diet failed apparently to damage the kidneys. The question of the protection against renal damage from ingested gelatin by the

<sup>1</sup> 'Delft' brand.

<sup>2</sup> 'Gold Label' brand.



TABLE 3

DIET NO.	NUMBER OF RATS	BODY WEIGHT	BODY LENGTH	BODY SURFACE	HEART WEIGHT		LIVER WEIGHT		KIDNEY WEIGHT				RATIO: BODY LENGTH BODY WEIGHT
					Actual	Per 100 sq. cm. body surface <sup>1</sup>	Actual	Per 100 sq. cm. body surface <sup>1</sup>	Mean	P. E.	Coef. Var.	per cent	
		gm.	mm.	sq. cm.	mg.	mg.	gm.	gm.	mg.	mg.			
1 A	25	170	192	348	640	184	8.42	2.42	185	1.4	6.0	0.89	
1 B	21	195	198	381	730	191	8.69	2.28	218	2.0	6.9	0.99	
1 C	29	159	189	332	644	194	9.07	2.73	289	1.8	5.0	0.84	
2 A	22	180	194	361	656	183	7.54	2.24	178	1.6	6.1	0.93	
2 B	24	110	172	259	434	168	5.12	1.98	235	2.5	7.6	0.64	
2 C	20	86	162	219	347	158	3.95	1.80	268	3.0	7.4	0.53	
3 A	20	138	186	301	516	171	7.78	2.58	202	2.5	7.6	0.74	
3 B	22	197	200	384	676	176	9.92	2.58	262	2.5	6.6	0.98	
3 C	20	133	181	295	533	181	8.52	2.89	307	2.3	4.9	0.74	
4 A	20	129	182	289	503	174	5.91	2.04	201	3.4	11.9	0.71	
4 B	22	173	193	351	648	184	9.16	2.61	322	4.8	10.4	0.90	
4 C	21	109	170	258	433	168	6.58	2.55	433	5.3	8.2	0.64	

<sup>1</sup> Body surface calculated by the formula of Carman and Mitchell ('26)—S = 11.36 W<sup>2/3</sup>.

addition of casein to the diet is now the subject of another investigation. In the present experiments the kidneys appeared normal when the rats were killed. Likewise there was no evidence of any renal pathology upon any of the other diets.

The data for the twelve experiments have been summarized in table 3. Among the incidental measurements the small livers in the egg albumin fed rats, particularly on the higher intakes, are noteworthy. On every diet the kidneys became larger when the amount of protein was increased. The kidney weights produced by the gelatin diets are far beyond any of the others and although this may be a specific physiological effect of the gelatin we are prone to believe that it is an early stage in the renal damage from gelatin that has been mentioned. True, the blood urea concentrations of these gelatin fed rats were not abnormal and by routine histological methods no changes were noted in their kidneys. However, the swelling of the epithelial cells which eventually goes on to necrosis is very difficult to discern in the early stages.

In table 4 under the 'A' columns the kidney weights as observed in relation to the body surface calculated by the formula of Carman and Mitchell ('26) have been compared with those calculated from the average protein intake (in relation to the same body surface figure) during the last 10 days of each experiment by means of the casein formula:

$$\text{renal weight} = (\text{protein intake} + 2.75) \div 0.0183$$

Both the egg albumin and the blood albumin diets apparently produced kidneys significantly larger than would be expected from the protein intakes. This extra enlargement may be a pseudo-enlargement due to our method of calculating body surface. Carman and Mitchell's formula was perfectly satisfactory for rats in good condition or where the body surface simply served as a reference standard for the kidney weight and food ingredients in a given group of rats as in earlier studies in this series. When it is desired to compare the relationship with the standard in diverse groups as in the present study, it may not be satisfactory. In our calculations

TABLE 4

GROUP NO.	PROTEIN	INTAKE PER 100 SQ. CM. BODY SURFACE PER DAY				MILLIGRAMS KIDNEY PER 100 SQ. CM. BODY SURFACE				DEVIATION OF OBSERVED FROM CALCULATED				RATIO: KIDNEY WEIGHT HEART WEIGHT
		Food		Protein		Observed		Calculated		A	B	B <sup>1</sup>	B <sup>2</sup>	
		A	B	A	B	A	B							
1 A	Casein	gm. 3.39	gm. 4.40	gm. 0.61	gm. 0.79	185	240	183	193	per cent 1.8	per cent 24.3			1.01
1 B	Casein	3.02	4.06	1.30	1.74	218	293	221	245	—1.4	16.4	17.6	0.0	1.14
1 C	Casein	3.71	4.76	2.49	3.19	289	370	286	325	1.5	12.2			1.50
2 A	Egg albumin	2.59	3.35	0.44	0.57	178	232	174	181	2.2	22.0			0.98
2 B	Egg albumin	2.32	2.83	0.95	1.15	235	284	202	213	14.0	25.0	19.9	2.3	1.40
2 C	Egg albumin	2.93	3.44	1.89	2.22	268	311	254	271	5.2	12.8			1.68
3 A	Blood albumin	2.14	2.62	0.36	0.44	202	247	175	174	7.8	42.0			1.18
3 B	Blood albumin	3.09	4.04	1.30	1.70	262	360	222	243	15.3	48.2	40.9	23.3	1.56
3 C	Blood albumin	3.68	4.54	2.44	3.04	307	420	284	317	10.8	32.5			1.70
4 A	Gelatin	2.53	3.07	0.60	0.73	201	246	183	190	9.0	29.5			1.16
4 B	Gelatin	3.29	4.26	1.43	1.85	322	418	229	251	28.8	66.6	56.0	38.4	1.76
4 C	Gelatin	3.42	3.67	2.19	2.35	443	478	270	278	39.2	72.0			2.65

REMARKS: A = Body surface calculated by the formula of Carman and Mitchell ('26). B = Body surface calculated by the formula of Lee and Clark ('29). B<sup>1</sup> = Averages for the three groups on each type of protein. B<sup>2</sup> = Averages for the three groups on each type of protein adjusted to the basis of zero deviation of observed from calculated.

body surface depends solely on body weight and the body surface:body weight relationship may be changed by variations in the amount of body fat which seemed to exist or severe stunting which is evidenced by the depressed body length—

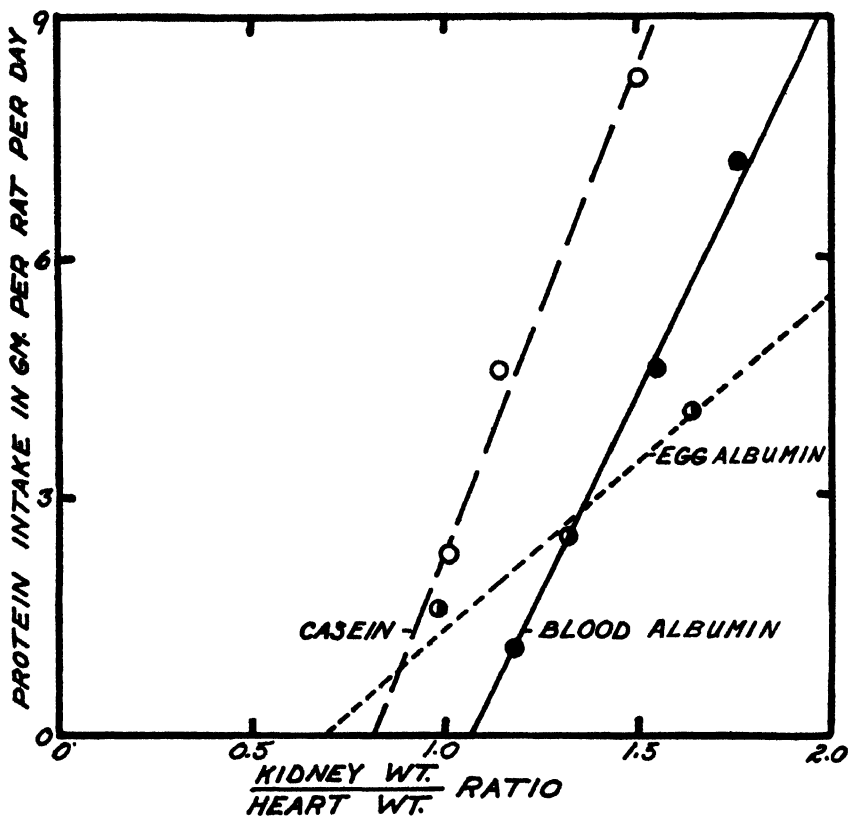


Figure 1

body weight ratios (Jackson, '24) in table 3. Lee and Clark ('29), in their rat body surface formula no. 4, have incorporated the nutritional correction factor used by Cowgill and Drabkin ('27) in their body surface formula for the dog. This formula makes a good correction for variations in body fat and the effects of severe stunting. It is:

$$S = 10.76 W^{0.61} \left( 0.310 \div \frac{W^{1/3}}{L} \right)$$

We have recalculated our data on this basis in table 4 under the 'B' columns. Since our protein-renal weight formula is based upon a different body surface even the casein diet groups show a definite deviation of the observed from the calculated kidney weights. When the figures are adjusted to a basis of no deviation for the casein groups we find that egg albumin has the same influence on renal weight as casein. Blood albumin appears to have a significantly greater effect upon the weight of the kidneys than either of the other two proteins.

There is one more possible manner of interpreting the data presented here. In another paper of this series will be considered the use of heart weight in place of body size measurements as a reference standard for the weight of the kidneys. Under some conditions it is useless while in others it seems to have considerable value. The present case is one of the latter. In figure 1 the ratio kidney weight  $\div$  heart weight has been charted against the protein intake per rat per day. The usual linear relationship is obvious although they diverge significantly from the casein formula. Blood albumin has a slightly greater and egg albumin a far greater influence on renal weight than protein fed as casein. This result is supported in general by the calculations on our old body surface figures.

#### SUMMARY

In male albino rats an increased protein intake in the form of egg albumin or blood albumin has a greater influence upon renal weight than protein in the form of casein. Gelatin produces a much larger increase in renal weight than any other protein source which has been fed. There is evidence that this may be of a pathological rather than a physiological nature.

## ADDENDUM

Shortly after this manuscript was submitted for publication a paper by Wilson (Wilson, H. E. C., *Biochem. J.*, 1933, vol. 27, p. 1348) on the same subject appeared. He reports that gelatin produces a much greater increase in renal weight than other proteins, a finding which we confirm. Wilson does not suggest that this may be of a pathological nature as we believe. His conclusion that glycine and glutamic acid nitrogen have practically the same influence as casein nitrogen coincides with our findings (Mac Kay, E. M., *J. Nutrition*, 1933, vol. 6, p. 157). This author's findings in connection with the addition of phosphate to the diet must be modified by the fact that phosphate renal enlargement is definitely (Mac Kay, E. M., and Oliver, J., *Proc. Soc. Exp. Biol. and Med.*, 1930, vol. 28, p. 324) pathological.

## LITERATURE CITED

- BOAS, M. A. 1927 The effect of desiccation upon the nutritive properties of egg white. *Biochem. J.*, vol. 21, pp. 712-724.
- CARMAN, G. G., AND H. H. MITCHELL 1926 Estimation of the surface area of the white rat. *Am. J. Physiol.*, vol. 76, p. 380-384.
- COWGILL, G. R., AND D. L. DRABKIN 1927 Determination of a formula for the surface area of the dog together with a consideration of formulae available for other species. *Am. J. Physiol.*, vol. 81, pp. 36-61.
- FINDLAY, J. M., AND R. O. STERN 1929 Syndrome in rat resembling pink disease in man. *Arch. Dis. Childhood.*, vol. 4, pp. 1-11.
- JACKSON, C. M. 1925 Inanition and malnutrition. P. Blakiston's Son & Co., Philadelphia, pp. 80-94.
- LEE, M. O., AND E. CLARK 1929 Determination of the surface area of the white rat with its application to the expression of metabolic results. *Am. J. Physiol.*, vol. 89, pp. 24-33.
- MAC KAY, E. M., AND L. L. MAC KAY 1931 a Factors which determine renal weight. VII. Protein intake and age (second paper). *J. Nutrition*, vol. 3, pp. 375-385.
- 1931 b Factors which determine renal weight. VIII. Protein intake and sex. *J. Nutrition*, vol. 3, pp. 387-394.
- MAC KAY, E. M., L. L. MAC KAY AND T. ADDIS 1928 Factors which determine renal weight. V. The protein intake. *Am. J. Physiol.*, vol. 86, pp. 459-465.
- 1928 Factors which determine renal weight. VI. Influence of age on the relation of renal weight to the protein intake and the degree of renal hypertrophy produced by high protein diets. *Am. J. Physiol.*, vol. 86, pp. 466-470.
- PARSONS, H. T. 1931 The physiological effects of diets rich in egg white. *J. Biol. Chem.*, vol. 90, pp. 351-367.



# THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY INDIVIDUAL NUTRIENT DEFICIENCIES <sup>1</sup>

## I. THE EFFECTS OF CYSTINE DEFICIENCY

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### TWO FIGURES

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### INTRODUCTION

The study to be discussed is the first unit in a program of investigations of the methods by which individual nutrients contribute to the specific effects of foods.

The program as a whole and the point of view are direct consequences of the studies on energy metabolism initiated by Armsby in 1902, carried on by him until his death in 1921, and continued since 1922, with many changes of understanding and methods, under the direction of E. B. Forbes.

The fundamental principle upon which this program of studies is based is the net energy conception, in the light of which the net energy useful in nutrition is the gross energy of the food minus the several energy losses and expenses of food utilization.

The principal losses are the energy of the feces and urine, and, in the case of ruminants, the energy of the methane produced by fermentation of carbohydrates; and the energy expenses of food utilization are the work of prehension, mastication, deglutition, fermentation, digestion, peristalsis, ab-

<sup>1</sup> Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, January 8, 1934, as Technical Paper no. 627.



sorption, transportation, stimulation, synthesis, secretion and excretion.

This simple conception, that gross energy minus expense and loss of utilization equals net energy, has been most attractive, since it has seemed to offer the promise of a fundamentally valid system of measurements of energy values of foods, which would be serviceable as the basis of practical standards of nutrition; and the tables of starch equivalents computed by Kellner ('05), and of net energy values, either experimentally determined or computed by Armsby ('17), were founded upon this idea.

Later developments within the field of nutrition, however, have raised questions as to the practicability of net energy values of individual foods; have revealed them as remainders—varying in response to many influences—rather than as directly determinable constants; and have led this institute to undertake the analysis, which has been in progress for several years, of the whole subject of conditions affecting the utilization of food energy.

In this relation Forbes ('33) recently formulated the law of maximum normal nutritive value, a principle which was by no means new, but which had not been as clearly appreciated nor as generally recognized as it should be, in research in nutrition. This principle signifies, in most relations, that individual foods express their full nutritive value only as they are presented in nutritively complete and perfect, and quantitatively sufficient diets.

In this same light Forbes observed, several years ago ('29), that any deficiency of any essential nutrient must eventually affect the energy value of a ration.

Such sweeping postulates, however, require a deal of proving; hence the undertaking of the series of studies of which the present is the first to reach publication, the object of which is to determine the effects of deficiencies in the supply of single essential nutrients, other than as used for energy production, on the energy value of diets.

The immediate objective of the present study was to compare two diets of the same gross energy value, and of the

same composition, except for a difference in the quantity present of the amino acid cystine.

The criteria on which conclusions depend are the results of digestion, metabolism, and growth experiments—involving the determination of the nitrogen and the gross energy of the food, the feces, the urine and the body increase.

The first important work on the biological significance of the amino acid cystine was that of Osborne and Mendel ('15), who demonstrated that the addition of cystine to casein notably lowered the casein requirement for normal growth of rats.

Lewis ('17) found that a small quantity of cystine added to a diet which was poor in protein (supplied as beef heart) favorably influenced nitrogen retention by dogs.

Sherman and Merrill ('25) reported that cystine is also the first limiting amino acid as affecting the utilization of whole milk protein.

Mitchell and Beadles ('30) demonstrated that a diet containing 8 per cent protein (from skim milk powder) is definitely improved by the addition of 0.24 per cent of cystine, as determined by increase in live weight and in nitrogen retention. They did not determine the gross energy of the rat bodies, but the weight of the ether extract of the bodies indicated that in the case of at least one pair of rats among nine the energy stored by the rat which received the cystine-supplemented diet was less than that stored by its pair mate.

#### EXPERIMENTAL SUBJECTS

The subjects of the experiments were albino rats, approximately 28 days of age, and selected to weigh as nearly as possible 40 gm. each.

Ten such rats were killed and analyzed, at the beginning of the experiments, to serve as controls, by representing the initial status, as to nitrogen and energy content, of the rats which were fed.

The nitrogen content of the control rats averaged 25.2 mg. per gram of live weight (coefficient of variation = 3.4 per cent); and the energy content per gram of live weight averaged 1.50 Cal. (coefficient of variation = 8.4 per cent).

Expressed on the basis of empty weight (contents of alimentary tract removed), the values of nitrogen and energy per gram were 27.7 mg. and 1.65 Cal., respectively.

The 'fill' (alimentary content) of the control rats averaged 3.66 gm. per rat, the empty weight being 90.85 per cent of the live weight. The computation of the initial nitrogen and energy content of the experimental rats may obviously be made from either the live weights or the computed empty weights with identical results.

#### A PRELIMINARY EXPERIMENT

In a preliminary experiment in which two groups, of five rats each, were individually fed equal quantities of two rations, one cystine-deficient and the other cystine-supplemented, during a period of 10 weeks, the energy gains by the rats which received the cystine-supplemented diet averaged 14.1 per cent of the gross intake, while the energy gains of the other group averaged 10.6 per cent of the food energy. No difference in the digestibility of the dry matter of the rations of the two groups of rats was found. Since the constituents of the urine were not determined, no values for metabolizable energy and heat production were obtained. The average gain in live weight of the rats which received the cystine-supplemented diet was 71 gm., while that of their pair mates, which received a low-cystine diet, was 50 gm.

#### A SECOND EXPERIMENT

Following this preliminary test it was determined to conduct a second experiment with the same rations, with a larger number of rats, and to measure also the energy in the feces and urine, thus to learn, more in detail, the differences which characterize the energy utilization of the amino-acid deficient diet.

Ten pairs of weanling rats were selected for this experiment, and were fed for 14 weeks by the paired-feeding method, which was originally proposed by Armsby, but has been advocated of late especially by Mitchell (Mitchell and Beadles,

'30). All pair mates were of the same sex, of the same litter, and of approximately the same live weight.

#### METHOD OF FOOD ASSIGNMENT

Throughout the experiment the quantity of food given to each pair was determined as the quantity consumed, *ad libitum*, by the rat which ate the less; and the intake of the rats of each pair was exactly equalized at the end of each week.

This procedure has the effect to place the animals which receive the more efficient ration at a certain disadvantage as compared with those which receive the less efficient one, in that no food allowance is made for the maintenance of the additional body substance produced. This objection to equalized food intake, however, is pertinent only when the rats of a pair have diverged appreciably in live weight, at which time the difference in the quality of the rations compared has been demonstrated.

Maynard and associates ('32) have discussed this matter of food allowance in the use of the paired-feeding method at some length, and have followed Armsby in making food allowance for the maintenance of the extra gain produced on the more efficient rations, rather than Mitchell, who has modified the method of Armsby, in applying it to nutritional experiments with rats, by feeding the rations to be compared in equal quantities, regardless of difference in gains produced.

In the opinion of the writers the point of view of Armsby and of Maynard and associates is unquestionably correct, and they concede the argument of Maynard and associates that "it would seem theoretically sound in using growth as a measure to endeavor to equalize the food available for this function." Armsby, however, proposed the method for use in experiments with cattle, on rations not far from normal, while the present writers have used it in experiments with rats, on rations some of which are so far from normal as to result in physiological conditions distinctly different from those which Armsby had in mind; and the writers feel that

it would be more advisable to feed equal quantities of the rations to be compared, even though this procedure should submit the animals on the more efficient rations to a certain technical disadvantage, than to attempt exactly to equalize the quantities of food supplied in excess of the maintenance requirement, for the reason that there is no established basis of computation of the maintenance requirements of growing rats, on either normal or deficient diets.

Also, it seems questionable whether the quantity of food which just prevents a young rat from growing could be considered to be the quantity which it uses for maintenance when on full feed.

Further, Lusk ('28) has repeatedly pointed out that undernutrition may markedly diminish the basal metabolism (which constitutes the larger part of the maintenance requirement); and it is quite conceivable that a rat on a restricted food intake may use a part of its food energy for growth which could be considered as used for maintenance when the animal is on full feed; though, strictly speaking, maintenance requirements can be measured only on a maintenance plane of nutrition, and the significance of such measurements with reference to other planes of nutrition must rest upon assumption alone.

Again, growth may be diminished by deficiency of a single essential nutrient, though the energy intake exceeds the maintenance requirement; and it is by no means certain, in advance of experimental determination, whether the effect of a given nutritive deficiency will be to increase or to decrease the maintenance requirement.

In giving both animals of a matched pair the same quantity of food (in a comparison of diets differing in nutritive value), regardless of difference in gain in weight, it is true that as they diverge in weight, in response to the differences in the rations, the smaller animal will come to receive more food in relation to its body weight than will the larger animal, which receives the more efficient ration; and, at first thought, it would appear that the larger animal comes to be at a comparative disadvantage on this account; but it is necessary to

bear in mind the fact that the nutritive requirements of the animals, on the two treatments, differ reversely as the efficiency of the rations, and that whatever the theoretical inequity experienced by the larger animal it has become the larger because it is actually the more liberally fed of the two.

If the metabolizable energy of the two diets is essentially unaffected by the difference in nutritive value—as is likely to be the case—the difference in the gain of the two animals may be considered as an expression of the fact that with the more deficient diet the sum of the maintenance requirement of energy and the heat increment (that is, the heat loss) is greater than with the more efficient diet.

It seems not to be possible to compare two diets on a perfectly equitable quantitative basis unless both are nutritively complete and perfect.

The method of the present experiment, obviously, affords no basis for separation of the maintenance requirement of energy and the heat increment (specific dynamic effect). These two items will therefore be considered together, as the heat production or the heat loss. It may be well to observe, however, that the maintenance requirement of energy is a practical measure which in order to be significant in relation to the utilization of a diet for body increase, as in the present investigation, or in other practical relations, must be determined under conditions as nearly as possible those to which it is to apply. It is affected not only by the weight of the animal but also by its physiological condition—by its nutritive reserves—and is unaffected by the diet only as it may be assumed that it remains unchanged while the entire increase in heat production accompanying the use of food is considered to be energy expense of food utilization.

It is the belief of the writers, therefore, that the maintenance requirements of growing rats cannot be computed, on the basis of present understanding, with such accuracy as would make it proper to employ the results as a basis for food assignment in critical nutritional research.

The general method of paired feeding, however, is especially valuable in critical nutritive comparisons in that it yields results which can properly be subjected to unbiased statistical evaluation.

#### THE EXPERIMENTAL DIETS

The cystine-deficient diet was made up as follows: skim milk powder, 23.50 per cent; Osborne and Mendel salt mixture, 4.00 per cent; butter fat, 15.00 per cent; sucrose, 10.00 per cent; cellu flour, 4.00 per cent; NaCl, 1.00 per cent; starch, 41.50 per cent; and irradiated yeast, 1.00 per cent.

The cystine-supplemented diet contained 0.24 per cent added l-cystine, obtained from the Eastman Kodak Co., the skim milk powder being diminished to 22.99 per cent, and the starch increased to 41.77 per cent. The food was weighed out daily with a chemical balance, and a record kept of the quantities of food refused.

In an experiment concerned with cystine deficiency it is of interest to know the approximate cystine content of the rations, and in the present study the cystine of the unsupplemented diet was contained in skim milk powder and yeast.

Vickery and White ('33) recently presented results from which it is assumed that casein contains 0.22 per cent, and lactalbumin 2.56 per cent of cystine.

Assuming that milk contains 2.95 per cent casein, 0.52 per cent lactalbumin, and 0.05 per cent globulin (L. A. Rogers, associates of, '28), the protein of milk may be divided as follows: casein, 83.81 per cent; lactalbumin, 14.77 per cent; globulin, 1.42 per cent.

The cystine-deficient ration contained, by analysis, 8.54 per cent protein. The cystine content of the yeast used in the ration may be considered to be about 0.270 per cent.<sup>2</sup> The yeast constituted 1 per cent of the ration, and thus furnished about 0.524 per cent of protein and 0.003 per cent cystine in the ration.

<sup>2</sup> According to a private communication from F. A. Csonka.

On the basis of the data given the cystine contributed to the diet by the milk powder was 0.045 per cent, which value was increased by the yeast to a total of 0.048 per cent cystine in the entire diet.

In consideration of their yeast content alone the diets were deficient in vitamins B and G, though the skim milk powder doubtless contained some of these vitamins. During the fifth week of the experiment, the appearance of rats nos. 25 to 28, inclusive, indicated a possible vitamin B deficiency. One gram of yeast per week was therefore given to all rats during the sixth and seventh weeks, but was then discontinued because the rats on the cystine-deficient diet gained weight as rapidly as did their pair mates. A weekly feed consumption of 45 gm. signifies a cystine intake of about 0.022 gm. The above additional gram of yeast per week, which equalized the growth of pair mates, raised the cystine intake of the basal diet by about 12 per cent.

Although it has been known that yeast is rich in amino acids, including cystine, it has been frequently used as a source of vitamins, in studies of amino acid deficiencies (Jackson and Block, '32; Mitchell and Beadles, '30; Mitchell and Carman, '26; Rose, '31).

After the completion of the present experiment an article by du Vigneaud et al. ('32) appeared in which the yeast in a low-cystine diet was advantageously replaced by a milk vitamin concentrate.

#### EXPERIMENTAL CAGES

The rats were kept in individual cages (fig. 1), so equipped that very satisfactory separate collection of feces and of urine was accomplished. The main part of the cage fitted into an 8-inch crystallizing dish and was supported with its lower edge about  $1\frac{1}{2}$  inches below the edge of the dish by means of three wires soldered to the cage in such manner as to support it from the edge of the dish. The floor of the cage, about  $\frac{3}{4}$  inch below the edge of the dish, was made of  $\frac{1}{2}$ -inch mesh galvanized screen, and about an inch below the floor of the



cage an 8-mesh stainless steel wire screen served to collect the feces, and to allow the urine to drop through into the dish.

The top of this cage is closed when in use, by an inverted pie pan.

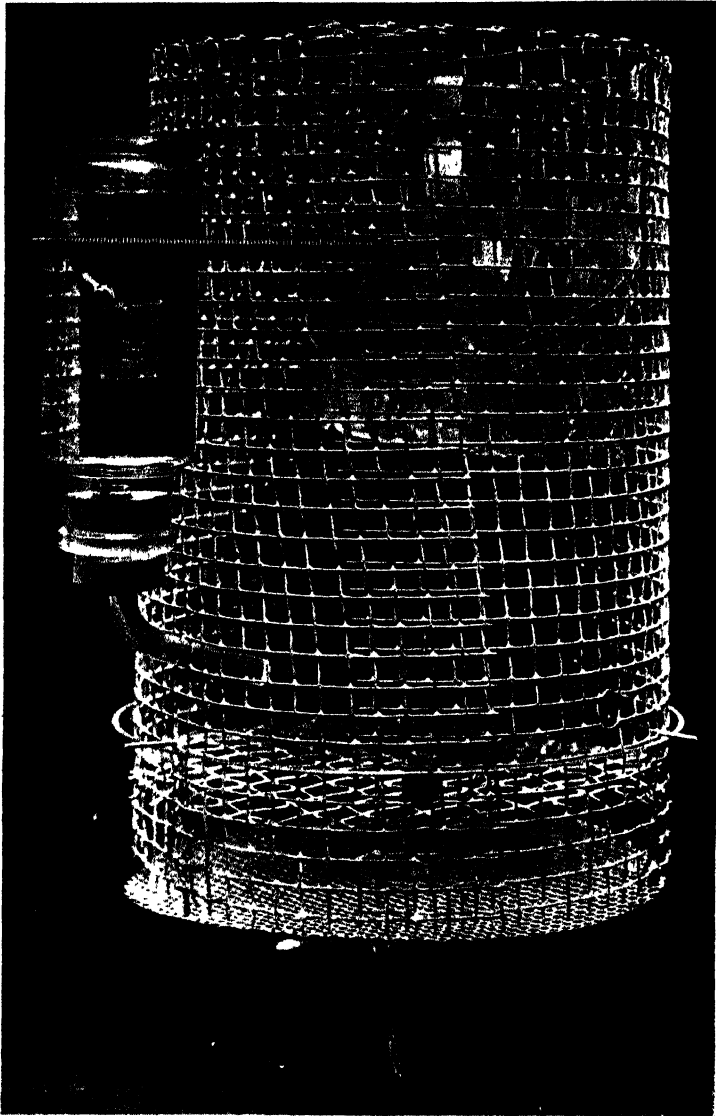


Fig. 1 A metabolism cage for rats.

The feeders, as shown in the figure, were constructed of galvanized iron, and were suspended from the upper edge of the cage by strips of the same material as that of which they were made.

A 50-cc. beaker was used as the feed container, this being held in place in the middle of the feeder by a close-fitting galvanized iron cup soldered to the floor of the feeder. The beaker was held in this galvanized iron cup by strips of sheet copper soldered to the edge of the cup and bent down over the edge of the beaker.

In order to prevent the rat from scooping the food out of the beaker a loose ring of galvanized iron, across which were soldered two wires at right angles to each other, was placed on top of the food. This device accomplished the intended purpose and should be valuable with unpalatable diets, but in the present experiment was found to be usually unnecessary.

This feeder was virtually perfect in preventing the scattering of food.

#### DAILY ROUTINE

The feed cups were removed from the cages each day at 8 A.M. and replaced at 4 P.M. This interval without food caused the rats to eat more eagerly, and it also helped, at the start of the experiment, to prevent the animals from forming the habit of depositing feces or urine in the feed cups. The feces were removed from the screens daily and kept in loosely stoppered bottles at room temperature.

The dishes and screens were washed with boiling water daily; the individual washings were made up to volume (250 cc.), and the solution was filtered and sampled for analysis, two aliquots being taken, one (50 cc.) for nitrogen and one (100 cc.) for carbon and energy determinations.

The aliquot for nitrogen determination was put into a 2½-liter bottle, with 15 cc. concentrated  $H_2SO_4$  as a preservative, the nitrogen being determined on the accumulated aliquots when the bottle had become nearly full.

No preservative was used in the crystallizing dishes. Any loss of nitrogen occurring during the intervals between the daily washings proved to be negligible, as shown by the satisfactory recovery of feed nitrogen in the feces, urine, and body gain, which averaged 98.4 per cent for the twenty rats, with a coefficient of variation of only  $\pm 1.43$ . The aliquots for energy and carbon determination were placed in pyrex dishes and dried in an oven, equipped with a fan, at a temperature of 50°C. One such composite was thus accumulated for each rat.

#### FINAL OBSERVATIONS

At the end of the experiment, energy, carbon, and nitrogen were determined in the dried urines. Losses of carbon and energy occurring during the process of drying were computed from the loss of nitrogen, on the basis of the assumption that the relationship between the energy and the carbon lost was as in urea. The percentages of carbon and energy lost during drying were found to average 4.2 and 5.7, respectively. The determined carbon and energy values of the urines were corrected for these losses.

At the end of 14 weeks the rats were killed by means of gas; the contents of the alimentary tract were removed and discarded; and the bodies were dried in vacuum desiccators. They were then exposed to room air for several days, weighed, cut up with heavy shears, and extracted with ether for 48 hours. The residue was again exposed to room air for several days, weighed, ground in a hand food chopper, until satisfactorily fine, and then subjected to analysis.

Energy determinations were made, on both the ether extract and the extraction residue, by means of the bomb calorimeter, the details of procedure being as described by Fries ('12).

This accounting for the energy of the entire bodies of the rats, in two samples, furnished an unusually accurate basis for measuring food utilization. Energy determinations were made in duplicate and were accepted only when the difference between repeats was less than 1 per cent.

## DISCUSSION OF RESULTS

In the course of this experiment there were 289 refusals of feed, 216 being by the cystine-deficient rats. This deviation of 71.5 from the mean, if chance only determined the result, is 8.41 times the standard deviation (8.50), and would occur by chance once only in millions of trials. Thus, the cystine-deficient rat was definitely the restricting member of the pair, with respect to food intake. This finding contrasts with that of Beadles et al. ('30) who found no significant difference in the number of feed refusals among cystine-deficient rats as compared with cystine-supplemented rats.

All the rats which received the cystine-supplemented ration made greater growth than did their pair mates, though in the case of pair no. 9 the difference was very small (chart 1). Among 140 comparisons of weekly gains, the cystine rat was favored 104.5 times, cases of equal gains being equally divided. The standard deviation of 140 events which may occur with equal probability in either of two ways is 5.92. The deviation of 34.5 from the expected outcome (70) if neither rat of a pair is favored, is 5.83 times the standard deviation, and would occur by chance once only in many thousands of trials (Fisher, '28). Furthermore, the superiority of the average weekly gain in weight of the cystine-supplemented rats as compared with their pair mates was 2.38 gm., as compared with 1.57 gm. for the few weeks during which the cystine-deficient rats gained more than their mates. The ratio of the gain in body weight to the weight of the dry matter of food eaten was 17.5 per cent for the cystine-deficient rats and 20.4 per cent for their pair mates (table 1).

Throughout the tables of results of this experiment all values represent single animals for 14 weeks, and the calorie values are large calories.

In table 2 it will be observed that in eight cases among ten the cystine-supplemented rat stored more energy than its pair mate, the average difference being 10.8 per cent of the smaller gain. A statistical study by 'Student's' method ('Student,' '08), reveals odds of 55 to 1 that the cystine-sup-

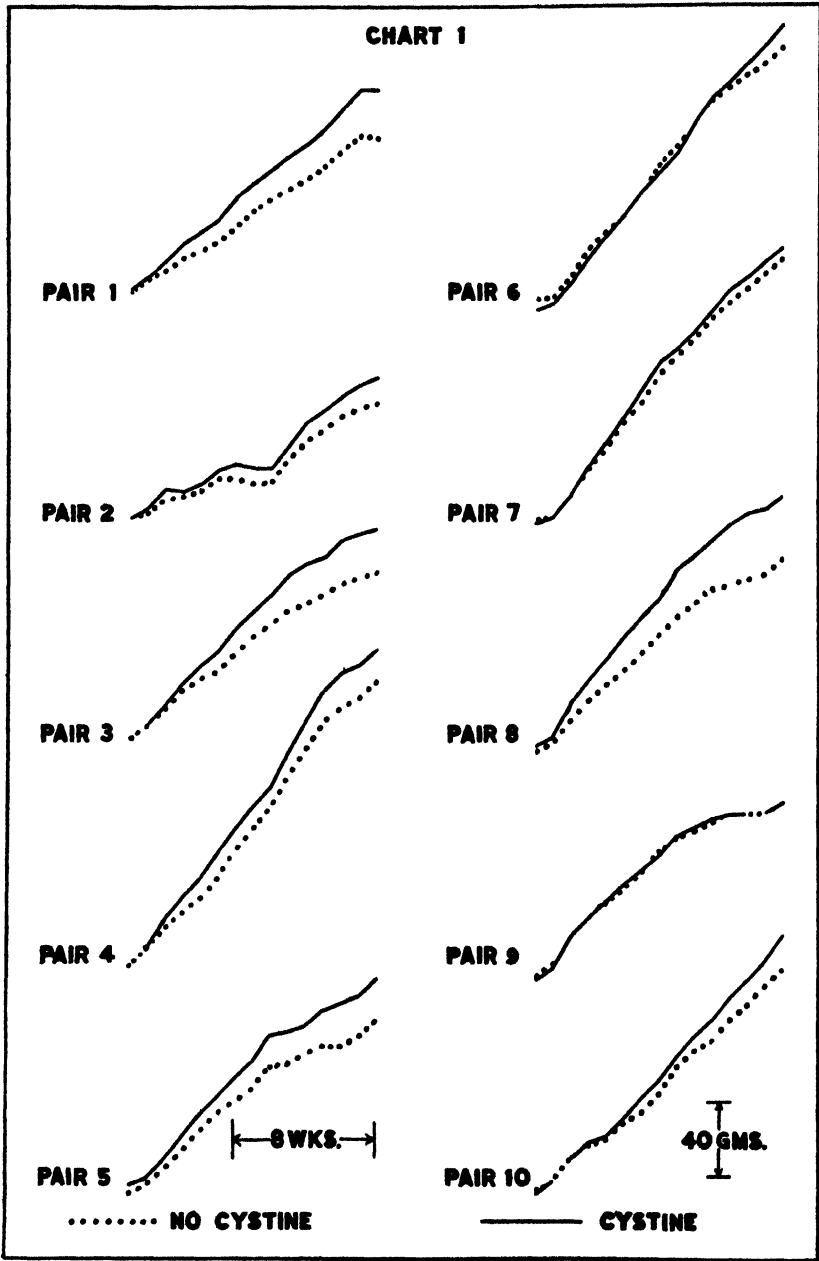


Chart 1 The growth of albino rats, during 14 weeks, as affected by cystine deficiency.

TABLE 1  
*Gain in body weight<sup>1</sup> related to dry matter of feed*

PAIR NO.	FOOD EATEN (DRY MATTER)	CYSTINE DEFICIENT DIET		CYSTINE SUPPLEMENTED DIET	
		Gain in body weight	Ratio of gain to D. M. of food	Gain in body weight	Ratio of gain to D. M. of food
	gm.	gm.	per cent	gm.	per cent
1	578.7	80.78	14.0	104.03	18.0
2	488.8	63.39	13.0	75.76	15.5
3	563.7	88.62	15.7	113.25	20.1
4	715.8	152.02	21.2	169.45	23.7
5	544.8	93.59	17.2	110.92	20.4
6	724.1	133.18	18.4	152.50	21.1
7	707.2	140.97	19.9	146.87	20.8
8	632.7	103.35	16.3	135.78	21.5
9	505.0	93.00	18.4	93.79	18.6
10	578.1	117.96	20.4	138.35	23.9
Average	603.9	106.69	17.5	124.07	20.4

<sup>1</sup> Excluding contents of alimentary tract.

TABLE 2  
*Energy of body gain related to gross energy, and to metabolizable energy of feed*

PAIR NO.	FEED ENERGY	CYSTINE DEFICIENT DIET				CYSTINE SUPPLEMENTED DIET				
		Body gain		Metabo- lizable energy <sup>1</sup>	Body gain as percent- age of metabo- lizable energy	Body gain		Metabo- lizable energy <sup>1</sup>	Body gain as percent- age of metabo- lizable energy	Body gain of energy of 'no cystine' rat as percentage of gain of pair mate
		Cal.	Per cent of feed energy			Cal.	Per cent of feed energy			
	Cal.	Cal.		Cal.	per cent	Cal.		Cal.	per cent	per cent
1	2770	240.4	8.7	2329.9	10.3	333.8	12.1	2400.0	13.9	72.0
2	2340	234.3	10.0	2000.0	11.7	255.1	10.9	2019.6	12.6	91.8
3	2698	319.2	11.8	2371.1	13.5	406.4	15.1	2394.4	17.0	78.5
4	3427	596.9	17.4	2988.6	20.0	586.4	17.1	2968.6	19.8	101.8
5	2608	367.9	14.1	2281.1	16.1	375.5	14.4	2280.6	16.5	98.0
6	3467	493.1	14.2	3012.1	16.4	552.8	15.9	3024.5	18.2	89.2
7	3386	431.6	12.7	2987.5	14.4	462.8	13.7	2969.6	15.6	93.3
8	3029	333.7	11.0	2615.0	12.8	478.5	15.8	2651.5	18.0	69.7
9	2418	369.4	15.3	2131.1	17.3	265.5	11.0	2114.2	12.6	139.1
10	2768	352.6	12.7	2424.4	14.5	426.3	15.4	2432.3	17.5	82.7
Average	2891	373.9	12.8	2514.1	14.7	414.3	14.1	2525.5	16.2	91.6

<sup>1</sup> Uncorrected for non-metabolizable energy of protein gained.

plemented diet produced greater energy gains than the cystine-deficient diet.

In two cases, however (pairs nos. 4 and 9) the cystine-deficient rats stored more fat and more energy than did their pair mates (table 4). These two rats also made more efficient utilization of metabolizable energy for body gain, thus showing that gain in live weight may not accurately indicate the efficiency of utilization of feed energy.

A smaller quantity of cystine in the deficient ration might conceivably have reversed the result with pair no. 4, but in the case of pair no. 9 it must be concluded that the cystine-deficient rat was definitely superior to its pair mate in its inherent capacity to synthesize and to store fat. It is perhaps significant in this relation that the rat of pair no. 9 which received the cystine-supplemented diet was the smallest individual among the twenty used in the experiment. The small initial weight of the rat (31 gm.), considered together with the inferiority of its energy gain on the cystine-supplemented diet, suggests that this individual was physiologically inferior to its mate.

The metabolizable energy of the two rations was, on an average, essentially alike (table 2).

The energy of the urine averaged 63.4 Cal. for the cystine-deficient rats, and 60.6 Cal. for the cystine-supplemented rats, this difference being 4.62 per cent of the smaller quantity. Although in nine cases out of ten the energy loss in the urine was greater with the cystine-deficient rat than with the pair mate, the odds are only 18 to 1 that the cystine-deficient diet was responsible for the greater loss of energy in the urine. In the case of pair no. 4 the energy of the urine of the cystine-deficient rat was 11.6 Cal. lower than that of its pair mate.

The energy of the feces (table 5), which is also involved in the measurement of metabolizable energy, was, on an average, 8.6 Cal. higher for the cystine-deficient rats, but with odds of only 8 to 1 that this finding did not occur by chance. This energy loss in feces, therefore, was 2.74 per cent less with the rats which received the cystine-supplemented diet.

The heat production (table 3), including the maintenance requirement and the heat increment due to the food, was obtained by subtracting from the gross energy of the food that of the feces, the urine, and the body gain. Although the differences were small, the greater heat loss occurred, in nine cases among the ten, with the cystine-deficient rat, the exception being, again, with pair no. 9. The average difference in heat production was 1.35 per cent less with the rats which received the cystine-supplemented diet, the significance of this difference being expressed by odds of 38 to 1.

TABLE 3  
*Heat loss related to energy of feed*

PAIR NO.	FEED ENERGY	CYSTINE DEFICIENT DIET		CYSTINE SUPPLEMENTED DIET	
		Heat loss		Heat loss	
			Per cent of feed energy		Per cent of feed energy
	Cal.	Cal.		Cal.	
1	2770	2089.5	75.4	2066.2	74.6
2	2340	1765.7	75.5	1764.5	75.4
3	2698	2051.9	76.1	1988.0	73.7
4	3427	2391.7	69.8	2382.2	69.5
5	2608	1913.2	73.4	1905.1	73.0
6	3467	2519.0	72.7	2471.7	71.3
7	3386	2555.9	75.5	2506.8	74.0
8	3029	2281.3	75.3	2173.0	71.7
9	2418	1761.7	72.9	1848.7	76.5
10	2768	2071.8	74.8	2006.0	72.5
Average	2891	2140.2	74.1	2111.2	73.2

The 40.4 Cal. (10.8 per cent) difference in energy gain, in favor of the rats which received the cystine-supplemented diet, comprised 29 Cal. difference in heat, 8.6 Cal. in feces and 2.8 Cal. in urine. The difference in heat may be allocated, by definition, either to the heat increment due to the food or to the maintenance quota.

Thus, the important difference in the energy value of the rations was not digestibility, nor in metabolizability, but in the proportion of the metabolizable energy which was utilized for body gain.



Table 2 shows the energy of the body gain as percentage of the metabolizable energy, the energy gain of the rats on the deficient diet being 91.6 per cent as much as that of their pair mates on the cystine-supplemented diet. If pair no. 9

TABLE 4

*Nitrogen of body gain related to fat and energy of body gain and to nitrogen of feed*

PAIR NO.	NITROGEN OF BODY GAIN	FAT GAINED		ENERGY GAINED			NITROGEN OF FEED	
			Per gram nitrogen gained		As protein	As fat		Utilized for body gain
Cystine deficient diet								
	gm.	gm.	gm.	Cal.	per cent	per cent	gm.	per cent
1	2.56	15.4	6.0	240.4	39.1	60.9	8.55	29.9
2	1.88	17.5	9.3	234.3	29.3	70.7	7.24	26.0
3	2.57	23.7	9.2	319.2	30.3	69.7	8.33	30.9
4	3.97	48.9	12.3	596.9	23.1	76.9	10.54	37.7
5	2.42	30.1	12.4	367.9	22.8	77.2	8.02	30.2
6	3.74	38.4	10.3	493.1	26.6	73.4	10.63	35.2
7	4.23	30.0	7.1	431.6	35.0	65.0	10.38	40.8
8	3.13	23.9	7.6	333.7	33.1	66.9	9.30	33.7
9	2.60	29.7	11.4	369.4	24.9	75.1	7.45	34.9
10	3.45	25.7	7.4	352.6	33.2	66.8	8.51	40.5
Average	3.06	28.3	9.3	373.9	29.7	70.3	8.90	34.0
Cystine supplemented diet								
1	3.48	22.6	6.5	333.8	35.9	64.1	8.55	40.7
2	2.30	18.0	7.8	255.1	32.9	67.1	7.24	31.8
3	3.37	30.3	9.0	406.4	29.7	70.3	8.33	40.5
4	4.93	44.3	9.0	586.4	28.8	71.2	10.54	46.8
5	3.40	27.8	8.2	375.5	30.5	69.5	8.02	42.4
6	4.45	42.9	9.6	552.8	27.2	72.8	10.63	41.9
7	4.60	32.6	7.1	462.8	34.4	65.6	10.38	44.3
8	4.01	35.9	9.0	478.5	29.3	70.7	9.30	43.1
9	3.17	16.7	5.3	265.5	42.1	57.9	7.45	42.6
10	4.16	29.8	7.2	426.3	34.7	65.3	8.51	48.9
Average	3.79	30.1	7.9	414.3	32.6	67.4	8.90	42.3

is omitted the average energy gain from the deficient diet is diminished to 86.3 per cent of that from the cystine-supplemented diet.

It may be seen from table 4 that in every case the cystine-supplemented rat synthesized more body protein than its

pair mate, the average difference being 24 per cent; and, likewise, without exception, eliminated less nitrogen in the urine. No consistent difference was found in nitrogen elimination through the feces.

The ratio of carbon to nitrogen in the urine, as explained by Bickel and Kauffman ('25), is considered to have a definite significance in the metabolism of human beings. They found the ratio to increase in cases of avitaminosis, due to an increased excretion of carbon compounds in the urine.

Bickel and Remesow ('27) noted the influence of the composition, especially the amino acid content, of food, on the C to N ratio in the urine. They found that when certain food protein was in part replaced by an amino acid, additional nitrogen was retained without there being increased oxidation of protein, which resulted in a higher C to N ratio in the urine.

It will be seen in table 6 that while the C:N ratios of pair mates do not differ significantly, the ratios are all very high, ranging from 1.6 to 2.2, the average for the rats receiving the cystine-supplemented diet being 2.0, and for their pair mates 1.8.

TABLE 5  
*Digestibility of protein and energy producing nutriment*

PAIR NO.	FEED NITROGEN	FEED ENERGY	CYSTINE DEFICIENT DIET				CYSTINE SUPPLEMENTED DIET			
			Nitrogen		Energy		Nitrogen		Energy	
			Feces	Digested	Feces	Digested	Feces	Digested	Feces	Digested
	gm.	Cal.	gm.	per cent	Cal.	per cent	gm.	per cent	Cal.	per cent
1	8.55	2770	2.04	76.1	366.5	86.8	1.85	78.4	307.9	88.9
2	7.24	2340	1.66	77.1	282.4	87.9	1.72	76.2	270.2	88.5
3	8.33	2698	1.77	78.8	267.2	90.1	1.48	82.2	244.8	90.9
4	10.54	3427	2.38	77.4	381.5	88.9	2.00	81.0	389.9	88.6
5	8.02	2608	1.51	81.2	268.1	89.7	1.63	79.7	275.2	89.4
6	10.63	3467	2.24	78.9	370.0	89.3	1.98	81.4	360.0	89.6
7	10.38	3386	2.06	80.2	326.8	90.3	2.05	80.3	345.0	89.8
8	9.30	3029	1.95	79.0	352.8	88.4	1.77	81.0	316.4	89.6
9	7.45	2418	1.37	81.6	233.5	90.3	1.48	80.1	254.5	89.5
10	8.51	2768	1.68	80.3	286.9	89.6	1.59	81.3	285.7	89.7
Average	8.90	2891	1.87	79.1	313.6	89.1	1.76	80.2	305.0	89.5

It is shown in table 4 that the cystine-supplemented rats, on an average, utilized 42.3 per cent of the food nitrogen for body gain, while the cystine-deficient rats utilized 34.0 per cent of their food nitrogen. In an unpublished experiment of this series, in which 17.7 per cent of protein was supplied by yellow corn and casein, there being no known nutritive deficiency in the ration, the utilization of food protein for body growth was only 23.7 per cent, the C:N ratio in the urine being 0.9. In a third experiment of this same series,

TABLE 6  
*Urinary nitrogen related to urinary carbon*

PAIR NO.	CYSTINE DEFICIENT DIET			CYSTINE SUPPLEMENTED DIET		
	Urinary carbon	Urinary nitrogen	Carbon-nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon-nitrogen ratio
	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	
1	7.75	3.79	2.0	6.29	3.33	1.9
2	5.95	3.70	1.6	5.12	3.28	1.6
3	6.01	3.63	1.7	5.90	3.27	1.8
4	8.00	4.19	1.9	7.32	3.46	2.1
5	6.26	3.82	1.6	5.56	2.97	1.9
6	9.54	4.41	2.2	8.74	4.05	2.2
7	7.29	3.76	1.9	7.41	3.44	2.2
8	6.32	4.06	1.6	6.57	3.37	1.9
9	5.62	3.39	1.7	5.27	2.65	2.0
10	6.03	3.22	1.9	5.42	2.50	2.2
Average	6.88	3.80	1.8	6.36	3.23	2.0

in which the complete diet contained 18 per cent protein (casein), 18.2 per cent of the food protein was utilized for growth, with a C:N ratio in the urine of 0.67. The amount of carbon in the urine from the three experiments was not greatly different; and the very high C:N ratios reported in this paper were determined primarily by the low percentage of nitrogen in the rations—about half of that required for optimum growth.

Table 4 also shows that with the rats on the deficient diet 29.7 per cent of the energy of the body gain was represented by protein, and 70.3 per cent by fat, while with the rats on

the cystine-supplemented diet 32.6 per cent of the gain was represented by protein, and 67.4 per cent by fat. In connection with the corresponding difference in utilization of food nitrogen these differences in composition of the body increase are considered significant.

The following statement by Peters and Van Slyke ('31) is pertinent to the finding of the small amounts of urinary nitrogen in this experiment: "The tendency for growth to continue in spite of all obstacles is so great that, provided the caloric value of the food is sufficiently great, the growing child will retain nitrogen if he is given anything in excess of the requirement for endogenous metabolism."

Sugar in the urine was not determined, and such high C to N ratios as were obtained might result from elimination of small quantities of sugar or other non-nitrogenous substance; but the C to N ratio of urea is so low that if the quantity of non-nitrogenous substance in the urine were little affected by the experimental treatment, the very low protein intake which prevailed, and the consequent low urea outgo, alone, would contribute prominently to the production of high C to N ratios. An indication that the high ratios of carbon to nitrogen found in this experiment result from the low protein intake is the finding of Forbes and associates ('33), who have reported ratios as high as 4.0, the values varying inversely as the amount of protein in the rations, none of which was known to be pathological.

#### SUMMARY

Twenty young growing rats were fed for 14 weeks, by the paired-feeding method, to demonstrate the ways in which cystine affects the utilization of food nitrogen and energy when added to a cystine-deficient diet.

The diets compared contained 8 per cent of protein, from skim milk powder; one diet was supplemented with 0.24 per cent of l-cystine.

Cystine deficiency unfavorably affected the appetite, but was without certain effect on the digestibility of the food protein.

The rats which received the additional 0.24 per cent of cystine made the greater growth, and stored 10.8 per cent more energy and 24.4 per cent more nitrogen than did the controls.

During the 14 weeks, the rats which received the cystine stored on the average 40.4 Cal. more than did the controls. This gain was accompanied by 29 Cal. less loss in heat, 8.6 Cal. less loss in feces, and 2.8 Cal. less loss in urine, by the rats which received the cystine supplement.

These differences in energy loss, when related to the gross energy of the diets, were 0.1 per cent as urine, 0.3 per cent as feces, and 1.0 per cent as heat.

The ratio of carbon to nitrogen in the urine was approximately 2.0, and did not differ significantly between the two groups. The unusual magnitude of this value was due to the low proportion of protein in the diet.

#### LITERATURE CITED

- ARMSBY, H. P. 1917 The nutrition of farm animals. Macmillan Co., New York.
- BEADLES, J. R., W. W. BRAMAN AND H. H. MITCHELL 1930 The cystine deficiency of the proteins of garden peas and of potatoes. *J. Biol. Chem.*, vol. 88, p. 615.
- BICKEL, A., AND O. KAUFFMANN 1925 Über das Wesen und die Beeinflussung aglykosurischer Stoffwechselstörungen der N-freien kohlenstoffhaltigen Substanz durch Insulin (Dysoxidative Karbonurie). *Chem. Zentr.*, II, 1876.
- BICKEL, A., AND I. REMESOW 1927 Über die Beeinflussung des Harnquotienten C:N durch die Nahrungszusammensetzung unter besonderer Berücksichtigung der Aminosäuren. *Biochem. Zeitschr.*, Bd. 186, S. 54.
- DU VIGNEAUD, V., R. DORFMANN AND H. S. LORING 1932 A comparison of the growth-promoting properties of d- and l-cystine. *J. Biol. Chem.*, vol. 98, p. 577.
- FISHER, R. A. 1928 Statistical methods for research workers. 2nd ed. Oliver & Boyd, Edinburgh and London.
- FORBES, E. B. 1929 The problem of determination of net-energy values of feeding stuffs. *Am. J. Physiol.*, vol. 90, p. 348.
- 1933 The law of maximum normal nutritive value. *Science*, vol. 77, p. 306.
- FORBES, E. B., W. W. BRAMAN AND MAX KRISS WITH COLLABORATION 1933 The associative effects of feeds in relation to the utilization of feed energy. *J. Agri. Res.*, vol. 46, pp. 753-770.

- FRIES, J. A. 1912 Adiabatic device for bomb calorimeter. *J. Am. Chem. Soc.*, vol. 34, p. 643.
- JACKSON, R. W., AND R. J. BLOCK 1932 The metabolism of cystine and methionine. The availability of methionine in supplementing a diet deficient in cystine. *J. Biol. Chem.*, vol. 98, p. 465.
- KELLNER, O. 1905 *Die Ernährung der landwirtschaftlichen Nutztiere*. Berlin.
- LEWIS, H. B. 1917 The metabolism of sulphur. II. The influence of small amounts of cystine on the balance of nitrogen in dogs maintained on a low protein diet. *J. Biol. Chem.*, vol. 31, p. 363.
- LUSK, G. 1928 *The science of nutrition*, 4th ed., p. 168. W. B. Saunders Co., Philadelphia and London.
- MAYNARD, L. A., R. C. BENDER AND C. M. McCAY 1932 Vitamin A and protein content of various fish meals. *J. Agri. Res.*, vol. 44, p. 591.
- MITCHELL, H. H., AND G. G. CARMAN 1926 The composition of the gains in weight and the utilization of food energy in growing rats. *Am. J. Physiol.*, vol. 76, p. 398.
- MITCHELL, H. H., AND J. R. BEADLES 1930 The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins. *J. Nutrition*, vol. 2, p. 225.
- OSBORNE, T. B., AND L. B. MENDEL 1915 The comparative nutritive value of certain proteins in growth, and the problem of the protein minimum. *J. Biol. Chem.*, vol. 20, p. 351.
- PETERS, J. P., AND D. D. VAN SLYKE 1931 *Quantitative clinical chemistry*. Vol. 1. Interpretations. The Williams & Wilkins Co., Baltimore.
- ROGERS, L. A., ASSOCIATES OF 1928 *Fundamentals of dairy science*. Chem. Cat. Co., Inc., New York.
- ROSE, W. C. 1931 Feeding experiments with mixtures of highly purified amino acids. I. The inadequacy of diets containing nineteen amino acids. *J. Biol. Chem.*, vol. 94, p. 155.
- SHERMAN, H. C., AND A. T. MERRILL 1925 Cystine in the nutrition of the growing rat. *J. Biol. Chem.*, vol. 63, p. 331.
- 'STUDENT' 1908 The probable error of a mean. *Biometrika*, vol. 6, p. 1.
- VICKERY, H. B., AND A. WHITE 1933 The use of cysteine cuprous mercaptide in the determination of cystine. *J. Biol. Chem.*, vol. 99, p. 701.



# DISAPPEARANCE OF VITAMIN C FROM THE ADRENALS OF SCORBUTIC GUINEA PIGS

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Szent-Gyorgyi ('28) has shown that the cortex of normal ox adrenals contains a substance which reduces a neutral 0.4 per cent silver nitrate solution. He identified it as being an hexuronic acid. Svirbely and Szent-Gyorgyi ('33) found that the hexuronic acid has marked antiscorbutic properties. They gave it the name of 'ascorbic acid.' King and Waugh ('32) have shown that crystalline vitamin C from lemon juice is identical with the 'hexuronic acid' Szent-Gyorgyi isolated from adrenals. We have previously reported ('33) our observation that the cortex of adrenals from guinea pigs having scurvy does not reduce 0.4 per cent silver nitrate solution.<sup>2</sup> Moore and Ray ('32), Svirbely and Szent-Gyorgyi ('33), and Harris and Ray ('33) have reported similar observations.

We have used Szent-Gyorgyi's silver staining reaction as a means of following the rate of disappearance of ascorbic acid from the adrenals during the onset of scurvy. Szent-Gyorgyi ('28) found that ascorbic acid from adrenals reduces iodine quantitatively. We attempted to adapt this method for determining the amount of ascorbic acid in the adrenals and to follow its rate of disappearance during the onset of scurvy. Since iodine reacts with other substances known to

<sup>1</sup>This article is taken from a dissertation presented by Arthur E. Siehrs to the Graduate School of Northwestern University, in partial fulfillment of the requirements for the degree of doctor of philosophy.

<sup>2</sup>Preliminary report presented before the Society for Experimental Biology and Medicine, Chicago, May, 1932.



be present in the adrenal, the method is not specific for ascorbic acid. The method determines the quantity of iodine reducing substances present. Harris and Ray ('32) have found that an iodimetric method for determining the ascorbic acid content of lemon and orange juice is unsatisfactory, since iodine reacts with other substances present in the fruit juices.

#### EXPERIMENTAL

*Rate of disappearance of ascorbic acid as indicated by silver staining reaction.* Guinea pigs weighing 150 to 160 gm. were fed oats and carrots for 8 days. On the eighth day, two were killed. The adrenals were removed. One from each animal was sliced and placed in a neutral 0.4 per cent silver nitrate solution for 15 minutes. The pieces were exposed to the light from a 115 watt Mazda bulb at a distance of 8 inches. Both the cortex and the medulla reduced the silver nitrate solution; however, the medulla reduced more slowly than did the cortex. The cut surfaces of the cortex of adrenals from normal guinea pigs turn very dark brown, almost black. Szent-Gyorgyi ('28) states that the medulla does not reduce neutral silver nitrate.

The remaining animals were placed on the scorbutic diet of Sherman, LaMer and Campbell ('22). The animals were killed at various intervals from the second to the twenty-second day of the diet period, and the reducing power of their adrenals was determined similarly. The data are given in table 1.

There is a noticeable decrease in the intensity of reduction in the cortex on the second day of the diet period. The intensity of reduction decreased progressively up to the sixth day. The color of the cut surface changes progressively from a very dark brown, almost black, to a reddish orange on the fifth day. The slight change in appearance on the fifth day may be due to the presence of a very small amount of ascorbic acid or some other reducing substance; or it may be due to the precipitation of the proteins by silver nitrate. In the table we refer to this as 1+ reduction. Szent-Gyorgyi ('28)

states that adrenin does not reduce neutral silver nitrate. After the sixth day, if any reduction takes place in the cortex, the extent is so small that it is scarcely evident. Also, after the sixth day, it was noticed that the capsule of the adrenals showed a more marked change in appearance than did the cut surface of the cortex. The animals did not begin to lose weight until after the ninth day. Autopsies showed no hemorrhagic areas about the joints and sternum, with but one exception. Beginning on the eighteenth day, two animals were

TABLE 1

NO. OF ANIMAL	DAYS OF SCORBUTIC DIET	INITIAL WEIGHT	WEIGHT CHANGE	EXTENT OF REDUCTION	
				Cortex	Medulla
		gm.	gm.		
1	0	216	+ 4	++++	++++
2	1	208	+ 12	+++	+++
3	2	170	+ 8	++	+++
4	3	160	+ 8	+	++
5	6	225	+ 7	++	+++
6	8	158	— 16	+	+++
7	10	180	+ 1	+	+++
8	16	172	— 29	+	++
9	18	236	— 16	+	+++
10	18, then fed 3 cc. orange juice for 2 days	192	— 24	+++++	+++++
11	18, then fed 3 cc. orange juice for 4 days	182	— 10	+++++	+++++

fed 3 cc. of orange juice daily by dropper. The animals were killed on the twentieth and twenty-second days, respectively, and their adrenals examined for reducing power toward silver nitrate. The sliced adrenals turned black in the silver nitrate solution. The intensity of reduction after orange juice feeding was greater than the reduction in the case of the guinea pigs which had been fed a normal diet. This shows that the cortex of the adrenals is still normal as it relates to the metabolism of ascorbic acid and that scurvy is not a condition in which the cortex is rendered permeable to ascorbic acid, permitting its loss from the body.

Five adult guinea pigs, weighing 380 to 420 gm. were fed a diet of oats and carrots for 2 weeks. They were killed and their adrenals were removed; one from each was sliced and placed in the silver nitrate solution according to the usual method. The cortex, medulla and capsule turned black. Adrenals from guinea pigs weighing 150 to 190 gm. under similar conditions gave a very dark brown but not a definite black. This indicates that the ascorbic acid content of the gland varies with the age of the animal.

*Rate of disappearance of reducing substances in adrenals as indicated by an iodimetric method.* Szent-Gyorgyi ('28) showed that ascorbic acid reacts quantitatively with iodine. An attempt was made to adapt this method to the determination of ascorbic acid in adrenals. Gluthathione, cysteine and thioneine react with iodine under the conditions of the analysis. We found that adrenin does not react with iodine under these conditions. Tunnicliffe ('25) has reported that iodine does not react with urea, uric acid, creatinine, glucose and fructose. Hence, the iodimetric method determines the amount of iodine reducing substances in the adrenals and not ascorbic acid alone.

Twelve guinea pigs, weighing 350 to 500 gm., were kept together in a large cage and fed a normal diet of oats and carrots supplemented with green vegetables. At varying time intervals during a 36-day period, one animal was removed to another cage and placed on the scorbutic diet. The length of time intervals was such that at the end of the experimental period, one animal had been on the scorbutic diet for 36 days, one for 1 day, and the other ten for periods varying from 1 to 36 days. Two within the same weight range were continued on a normal diet. These animals had been used in a previous study but had been allowed to recover. It has been shown that adult guinea pigs of this size and age are much more resistant to scurvy than young guinea pigs weighing between 150 and 250 gm. The left adrenal was always used for the determination. It was triturated with 10 cc. of 10 per cent trichloroacetic acid, filtered, washed, and made up to 50 cc. in

a volumetric flask. Twenty cubic centimeters of this extract was placed in an Erlenmeyer flask. Four cubic centimeters of 25 per cent potassium iodide, 1 cc. of concentrated hydrochloric acid, 0.5 cc. of 1 per cent starch solution, and a slight excess of 0.002 N iodine solution were added. This was allowed to stand for 3 minutes. The excess iodine was then

TABLE 2

NO OF ANIMAL	WEIGHT OF ADRENAL	CUBIC CENTIMETER 0.002 N IODINE SOLUTION	CUBIC CENTIMETER IODINE PER 0.01 GM. ADRENAL	CUBIC CENTIMETER IODINE PER 100 GM. BODY WEIGHT	DAYS ON SCORBUTIC DIET	SILVER NITRATE REDUCTION OF ADRENALS		
						Cortex	Medulla	
1	0.14	0.78	0.56	0.16	36	+	++	Mild scurvy with slight hemorrhagic joints
2	0.24	0.80	0.32	0.27	32	+	+++	All joints very hemorrhagic, severe scurvy
3	0.19	1.18	0.62	0.30	30	++	+++	All joints hemorrhagic, mild scurvy
4	0.22	1.05	0.48	0.26	27	++	++	All joints very hemorrhagic, severe scurvy
5	0.14	0.90	0.67	0.16	19	+	++	Very slight scurvy
6	0.17	1.12	0.66	0.24	16	++	+++	Very slight scurvy
7	0.16	1.25	0.80	0.22	10	++	+++	Mild scurvy
8	0.36	1.68	0.47	0.34	5	+	++	All joints very hemorrhagic, severe scurvy
9	0.21	0.98	0.47	0.15	3	++	+++	No symptoms of scurvy
10	0.24	1.45	0.60	0.23	1	++	+++	No symptoms of scurvy
11	0.18	1.48	0.82	0.26	0	+++	+++	Normal
12	0.22	1.45	0.68	0.27	0	++++	++++	Normal

titrated with 0.002 N sodium thiosulfate. The blank titration amounted to 0.12 cc. which was subtracted from the difference in titration values. The amount of material available for each determination was insufficient to permit a separate determination of glutathione. The data are given in table 2. Column 3 shows that there is a progressive decrease in the total iodine reducing substances in the adrenal with the onset of scurvy. Column 4 shows that there is a progressive de-

crease of iodine reducing substances in the adrenal per unit weight of gland after the tenth day. Column 6 shows that there is no significant change in iodine reducing substances in the adrenals compared to body weight throughout the period. The symptoms of scurvy of animals 1 and 8 differ from the usual experience in that animal 8 showed symptoms of scurvy in 5 days whereas animal 1 showed only a mild scurvy after 36 days. The iodine reducing substance in the adrenals of these animals per unit weight of adrenal is in accord with the data found for other animals manifesting comparable severity of symptoms.

#### DISCUSSION

Heretofore, scurvy has been defined in terms of certain characteristic gross symptoms, such as loss of weight, loosening of teeth, subcutaneous and intramuscular hemorrhages, and soreness and swelling of the joints, which appear, in the case of guinea pigs, after they are fed a scorbutic diet for 2 or 3 weeks. This work indicates that there is prompt and profound decrease in the ascorbic acid content of the body on an ascorbic acid-free diet. As a result of this, there is a profound disturbance of the metabolism of the animal with the development of the characteristic symptoms. The characteristic symptoms are, thus, secondary to the more subtle loss of ascorbic acid in the body. Apparently, ascorbic acid is of primary importance in the metabolism of connective tissue. Scurvy is not due to the loss of ability of the adrenals to store ascorbic acid, for even in severe scurvy the feeding of orange juice is followed by its prompt appearance in the adrenals.

The observation that ascorbic acid promptly disappears from the adrenals on a scorbutic diet and promptly reappears when returned to diet furnishes a basis for a method of assaying the vitamin C content of foods.

## SUMMARY

Ascorbic acid in the cortex of the adrenals of guinea pigs progressively disappears during the onset of scurvy. The silver nitrate staining reaction shows that there is a decrease after the animal has been on a scorbutic diet for but 1 day. Minimum staining with silver nitrate is obtained on the sixth day. The feeding of 3 cc. of orange juice to scorbutic animals is followed by the prompt reappearance of ascorbic acid in the cortex of the adrenals. An iodimetric method for the determination of the iodine reducing substances in the adrenals, which includes ascorbic acid, shows that there is a progressive decrease of these substances during the onset of scurvy.

## LITERATURE CITED

- HARRIS, L. J., AND S. N. RAY 1932 Vitamin C and the suprarenal cortex. I. Antiscorbutic activity of ox suprarenal. *Biochem. J.*, vol. 26, pp. 2067-2075.
- 1933 Vitamin C and the suprarenal cortex. II. Loss of potency of guinea-pig suprarenals in scurvy, with notes on a method for determining antiscorbutic activity (hexuronic acid) by chemical means. *Biochem. J.*, vol. 27, pp. 303-310.
- KING, C. G., AND W. H. WAUGH 1932 Isolation and identification and vitamin C. *J. Biol. Chem.*, vol. 97, pp. 325-331.
- MILLER, C. O., A. E. SIEHRS AND F. G. BRAZDA 1933 Glucuronic acid as a growth factor in guinea pigs. *Proc. Soc. Exp. Biol. Med.*, vol. 30, pp. 636-638.
- MOORE, T., AND S. N. RAY 1932 Vitamin C and hexuronic acid. *Nature*, vol. 130, pp. 997-998.
- SHERMAN, H. C., V. K. LAMER AND H. L. CAMPBELL 1922 The quantitative determination of the anti-scorbutic vitamin (vitamin C). *J. Am. Chem. Soc.*, vol. 44, pp. 165-172.
- SIEHRS, A. E., AND C. O. MILLER 1933 Disappearance of vitamin C from adrenals of guinea pigs having scurvy. *Proc. Soc. Exp. Biol. Med.*, vol. 30, pp. 696-698.
- SVIRBELY, J. L., AND A. SZENT-GYORGYI 1933 The chemical nature of vitamin C. *Biochem. J.*, vol. 27, pp. 279-285.
- SZENT-GYORGYI, A. 1928 Observations on the function of peroxidase systems and the chemistry of the adrenal cortex. *Biochem. J.*, vol. 22, pp. 1387-1412.
- TUNNICLIFFE, H. E. 1925 The occurrence and quantitative determination of glutathione in tissues. *Biochem. J.*, vol. 19, pp. 194-198.



# SPECTRUM ANALYSIS OF MILK ASHES

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Some months ago while spectrum analyses of water from different parts of the Rocky Mountain region were being made, it was noted that some of them contained strong traces of silver. The ashes of human tissues from individuals who had been drinking water containing silver also gave spectrum evidence of strong traces of silver. It was decided to investigate milk for this element and at the same time to note traces of other elements present since considerable attention is now being given to their biological significance.

Milk has been investigated in this way by other workers. Wright and Papish ('29) did not report silver as being present in cow milk and neither did Blumberg and Rask ('33). Zbinden ('31) noted its presence in human milk, but not in cow milk. The samples of milk examined by the first and second named workers were secured in Great Britain and various parts of the United States other than Colorado and whether or not the drinking water or feed the cows were consuming contained silver is not mentioned.

## MATERIAL

Samples of milk from twelve cows were examined. These cows were from three different localities, where the drinking water was known to contain silver. Pyrex glass flasks were acid cleaned, rinsed finally with double distilled water, and dried in an inverted position. Into the cleaned flasks was



forced the milk which did not come into contact with the milking hand or with the cleaned teat other than the orifice of the duct. The first two or three streams were not placed in the flasks. Clean paper (not cardboard) covers were then placed over the tops of the flasks and the milk was not allowed to touch the paper. Cork stoppers should not be used as some examined contained silver, some of which was extracted by cold distilled water.

#### METHOD OF INVESTIGATION

Not to exceed 5 cc. of milk at one time was placed in platinum or silicon dishes, slowly evaporated, and ashed at the lowest possible temperature over a Meeker burner in a room free otherwise from air currents, and after dust had presumably settled. No substance was added to promote the ashing, which occurs best with relatively thin layers of milk. The ash was then covered until used. Controls consisting of powdered graphite from a graphite electrode and a mixture of CaO and MgO, to which the same amounts of double distilled water were added and evaporated after exposure under similar conditions for the same length of time, gave no evidence of contamination by dust.

Samples of the two different drinking waters used by the three herds were evaporated in pyrex dishes over Meeker burners and the residue reserved for spectrum analysis.

Some of the alfalfa grown in the same region and which was being eaten by some of the cows was also ashed, nothing being added to promote the ashing.

The spectra recorded ranged from  $\lambda$  2300 A. U. to  $\lambda$  6800 A. U. and were secured by means of a Hilger E 34 medium sized spectrograph. Quartz lenses focused the image of the arc upon the slit of the spectrograph, the images of the incandescent poles not being allowed to fall on the slit.

For the recording of wave lengths lower than  $\lambda$  5000 A. U. Eastman spectroscopic plates, type III and type IV—O were used. For longer wave lengths, type III—H, for the CaF<sub>2</sub> band, type III—F, and type IV—F were used. Eastman

x-ray developer was used to get maximum density and contrast

A direct current arc was employed for exciting the spectra.

Graphite and copper electrodes of 6 mm. diameter were used in establishing the arcs, the lower being made positive to the upper for the passage of a direct current.

Acheson spectroscopic graphite electrodes permit the satisfactory recording of most of the wave lengths. Either 10 or 20 mg. of the substance to be arched were placed on top of the lower electrode or in a small hole made in the top surface. When 20 mg. of ash were placed in a hole, 13 amperes of direct current were used to heat up the ash promptly so as to liberate the easily volatilized silver and permit the recording of its wave lengths in 4 seconds, the optical system being so diaphragmed that overexposure was avoided. If the ash was placed directly on top of the graphite electrode, 7.5 amperes were used and sufficient time of exposure to the photographic plate given to record satisfactorily the spectrum for the duration of the burn whether 10 or 20 mg. were used, the optical system having been properly adjusted for this. In the latter case, a convenient amount of the ash was placed on top of the lower electrode, the arc established for 2 or 3 seconds, more ash added, the arc reestablished, and thus repeated until the full amount of either 10 or 20 mg. had been arched.

It was desirable because of the molecular bands produced when graphite electrodes are used that may mask certain wave lengths of some of the elements sought to also use copper electrodes. It was found that 3 to 4 amperes of current were insufficient and 7.5 amperes were used. The ash was placed only on top of the lower positive electrode and arched in the manner described in the latter part of the last paragraph. The spectra of the substances investigated had placed immediately above them the spectrum of the iron arc by means of a Hartmann diaphragm.

## RESULTS

The accompanying table gives the elements found to be present in the milk. The presence and absence of the same elements in the water and in the alfalfa are also recorded together with the inclusion of a few that gave negative or questionable evidence of their presence in the milk.

<i>Trace Elements</i>				
	<i>Milk</i>	<i>Alfalfa</i>	<i>Water no. 1</i>	<i>Water no. 2</i>
Al	+	+	+	+
Ba	+	+	+	+
B	+	+	+	+
Cr	+ in seven	+	+	+
Cu	+	+	+	+
F	—	—	+	+
Fe	+	+	+	+
Pb	+	+	+	+
Li	+	+	+	+
Mn	—	+	+	+
Mo	+	+	+	—
Ag	+	+	+	+
Rb	+	+	—	—
Si	?	+	+	+
Sr	+	+	+	+
Sn	+ in a few	+	—	—
Ti	+	+	+	+
V	+	+	+	+
Zn	+	+	+	—

<i>Larger amounts of elements</i>				
	<i>Milk</i>	<i>Alfalfa</i>	<i>Water no. 1</i>	<i>Water no. 2</i>
Ca	+	+	+	+
Mg	+	+	+	+
P	+	+	—	—
K	+	+	+	+
Na	+	+	+	+

Traces of silver were shown to be present in the milk from twelve cows drinking water containing silver in traces. Alfalfa fed to the same cows also contained silver. The lines recorded were  $\lambda$  3280.67 A. U., and  $\lambda$  3382.88 A. U. The owner of one of the herds stated that a cow drinks 80 or more liters of water and eats at least 9 kg. of dry alfalfa each day.

Molybdenum in traces was present in each milk, in the alfalfa, and in one of the two drinking waters. The wave lengths noted were  $\lambda$  3798.26 A. U.,  $\lambda$  3864.12 A. U.,  $\lambda$  3902.96 A. U.,  $\lambda$  3132.60 A. U.,  $\lambda$  3170.35 A.U. and  $\lambda$  3193.98 A.U. Molybdenum was not reported as being present in milk by the investigators mentioned above who used spectrographic methods. H. ter Meulen ('32) using a colorimetric method reported 0.14 to 0.03 mg. of molybdenum per kilogram of milk as well as in the same amounts of blood, bile, eggs, and sundry tissues.

Fluorine was sought in the milk because the drinking waters contained about 2 p.p.m. The  $\text{CaF}_2$  band was absent, although the plate recorded the presence of at least 0.02 per cent F. in synthetic test mixtures, confirming a similar former experience to be reported elsewhere. Bone, dentine, and tooth enamel of individuals who had been drinking this fluorine-containing water, contained more fluorine than did the same tissues from individuals who had been living on the Atlantic seacoast (Boissevain and Drea, '33).

Traces of other elements found in the milk and already reported upon by other investigators are aluminum, barium, boron, chromium, copper, iron, lead, lithium, rubidium, strontium, titanium, vanadium, and zinc. Tin was found in three or four of the milks and is questionably present. The presence or absence of silicon is questionable.

Manganese was absent from the milk, although strong traces were found in one of the waters as well as in the alfalfa, and weak traces in the other water.

Calcium, magnesium, phosphorus, potassium, and sodium were present in the usual large amounts.

#### SUMMARY

Silver and molybdenum are added to the list of 'trace' elements in cow milk as demonstrated by spectrographic methods.

Silver was found in the milk of each of twelve cows drinking water and eating alfalfa containing strong traces of the

same element. Molybdenum was present in the milk of twelve cows. Either the alfalfa, the drinking water, or both contained molybdenum.

Manganese, although present in the water and in the alfalfa, was not demonstrated to be present in the milk.

Fluorine, which was present in the water to the amount of 2 p.p.m. was not shown to be present, although the test synthetic material recorded F when present in as small concentration as 0.02 per cent.

An account is given of the other elements already reported upon by other investigators.

#### LITERATURE CITED

- BLUMBERG, H., AND O. S. RASK 1933 The spectrographic analysis of milk ashes. *J. Nutrition*, vol. 6, p. 285.
- BOISSEVAIN, C. H., AND W. F. DREA 1933 The spectroscopic determination of fluorine in bones, teeth and other organs in relation to fluorine in drinking water. *J. Dent. Res.*, vol. 13, p. 495.
- MEULEN, H. TER 1932 Distribution of molybdenum. *Nature*, vol. 130, p. 966.
- WRIGHT, N. C., AND J. PAPISH 1929 The inorganic constituents of milk. *Science*, vol. 69, p. 78.
- ZBINDEN, C. 1931 The infinitely small quantities of certain elements in milk and their detection by spectrographic method. *Lait*, vol. 11, p. 114.

# AN ADAPTATION OF THE PAIRED-FEEDING METHOD FOR THE DETERMINATION OF THE SUPPLEMENTARY VALUE OF PROTEINS<sup>1</sup>

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The determination of the supplementary value of proteins is at best beset with difficulties. This is particularly true when we are called upon to evaluate the proteins from such diverse materials as those from animal products, from seeds and from the vegetative portions of plants.

During recent years the paired-feeding method (Mitchell and Beadles, '30) has found considerable application in the determination of the amino acid deficiencies of proteins. In our own laboratory (Haag, '31) we have used this method with a considerable degree of satisfaction in showing that rations in which the proteins are supplied by alfalfa leaf meal are improved by the addition of l-cystine. In addition we have attempted to adapt the paired-feeding idea to a study of the supplementary value between the proteins of alfalfa leaf meal and those of wheat bran.

Briefly, the method used was as follows: Three rats from the same litter were matched for weight and sex. One member of the triplet was fed a ration containing alfalfa leaf meal as the source of protein; the second member was fed a ration containing wheat bran as the source of protein; and the third member was fed a ration consisting of equal parts of the alfalfa leaf meal and wheat bran rations. By trial, the per cent of wheat bran was adjusted to produce approximately

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the same rate of growth as that obtained with the alfalfa ration. An attempt was made to equalize the digestible dry matter, crude fiber, mineral and vitamin contents to an extent which it was hoped would eliminate these factors from further consideration in assessing the nutritive value of the proteins of these rations. The growth obtained on the mixed ration was compared with the average of that obtained on the alfalfa and wheat bran rations. If sufficient care has been exercised in equalizing the experimental conditions referred

TABLE 1  
*Composition of rations*

CONSTITUENTS	NO. 73	NO. 74	NO. 88	NO. 89
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Alfalfa leaves (1930)	50.0	—	—	—
Alfalfa leaves (1931)	—	—	50.0	—
Wheat bran	—	50.0	—	40.0
Sucrose	10.0	—	10.0	10.0
Dextrin	17.0	22.5	15.5	17.0
Alfalfa stem fiber	—	5.0	—	8.0
Na H <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O	2.0	—	1.5	—
CaCO <sub>3</sub>	—	1.5	—	2.0
NaCl	1.0	1.0	1.0	1.0
Yeast	—	—	2.0	2.0
Cod liver oil	1.0	1.0	1.0	1.0
Salad oil	—	—	19.0	19.0
Butterfat	19.0	19.0	—	—
Crude protein	11.0	6.6	11.7	5.3

to above, an increased rate of growth on the mixed ration may, with appropriate reservations, be taken as indicating a supplementary effect between the proteins of alfalfa and of wheat bran.

The alfalfa leaf meal and wheat bran rations were prepared as indicated in table 1. Ration 75 consisted of a mixture of equal parts of rations 73 and 74. Ration 90 consisted of a mixture of equal parts of rations 88 and 89. The digestibility coefficient of the dry matter of these rations was approximately 71 to 72 per cent. The apparent digestibility of the crude proteins of the alfalfa, wheat bran and mixed

rations was 63, 65 and 64 per cent, respectively. The crude fiber content of these rations was approximately equalized by the use of an alfalfa stem fiber preparation from which most of the proteins had been removed by acid-alkali extraction.

The food intake and growth data are summarized in table 2. In the last column the presence or absence of a supplementary effect is indicated by means of a plus or a minus sign, respectively. A supplementary effect is indicated for those triplets where the gain in live weight on the mixed ration was greater than the average of the gains on the alfalfa and wheat bran rations. It will be noted that this method of comparison indicates a supplementary effect in fourteen out of sixteen cases. More significant than the apparently slight supplementary effect between the alfalfa and wheat bran rations is the decided superiority of the proteins of wheat bran over those of alfalfa. This superiority cannot be explained on the basis of greater digestibility.

It is realized that the differences in gain obtained in these experiments are not large, that the energy intake levels are not as high as might be desired, and that the results must be interpreted with appropriate reservations. It does appear, however, that this method of attack promises to be useful in the study of problems of the nature described in this paper.

In conclusion, it may be stated that the results obtained to date (sixteen triplets) indicate that this method of attack promises to prove useful in the study of the nutritive value of the proteins of bulky vegetable and forage crops; that wheat bran proteins are superior to those of alfalfa leaf meal; that this superiority is not due to differences in apparent digestibility; and that there appears to be a slight supplementary effect between the proteins of alfalfa leaf meal and those of wheat bran.

#### LITERATURE CITED

- HAAG, J. R. 1931 The physiological effect of rations restricted principally or solely to the alfalfa plant. II. Cystine as a limiting factor in the nutritive value of alfalfa proteins. *J. Nutrition*, vol. 4, pp. 363-370.
- MITCHELL, H. H., AND J. R. BEADLES 1930 The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins. *J. Nutrition*, vol. 2, pp. 225-243.



**TABLE 2**  
*Growth and food intake data*

TRIPLAIT NO.	RATION NO.	TYPE OF RATION	INITIAL WEIGHT	FINAL WEIGHT	TOTAL GAIN	TOTAL FOOD	WEEKS	SUPPLEMENTARY EFFECT
			<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>		
1	73	Alfalfa	78	125	47	558	10	+
	74	Bran	78	141	63	555		
	75	Mixed	80	136	56	555		
2	73	Alfalfa	85	107	22	507	10	+
	74	Bran	82	127	45	496		
	75	Mixed	82	128	46	509		
3	73	Alfalfa	88	106	18	437	10	+
	74	Bran	82	112	30	449		
	75	Mixed	80	110	30	449		
4	73	Alfalfa	65	93	28	433	10	—
	74	Bran	65	95	30	432		
	75	Mixed	65	93	28	436		
5	73	Alfalfa	73	91	18	468	10	+
	74	Bran	73	100	27	457		
	75	Mixed	73	102	29	469		
6	73	Alfalfa	67	92	25	447	10	+
	74	Bran	67	95	28	451		
	75	Mixed	67	102	35	451		
7	73	Alfalfa	85	83	-2	411	10	+
	74	Bran	85	97	12	419		
	75	Mixed	85	100	15	419		
8	73	Alfalfa	86	92	6	460	10	+
	74	Bran	78	97	19	460		
	75	Mixed	78	102	24	460		
9	88	Alfalfa	90	108	18	270	5	—
	89	Bran	85	97	12	261		
	90	Mixed	85	97	12	263		
10	88	Alfalfa	83	98	15	235	5	+
	89	Bran	80	84	4	262		
	90	Mixed	80	96	16	229		
11	88	Alfalfa	66	86	20	198	4	+
	89	Bran	65	78	13	187		
	90	Mixed	63	86	23	188		
12	88	Alfalfa	63	73	10	152	4	+
	89	Bran	60	67	7	134		
	90	Mixed	60	77	17	153		
13	88	Alfalfa	106	141	35	455	7	+
	89	Bran	106	144	38	455		
	90	Mixed	106	153	47	456		
14	88	Alfalfa	97	133	36	454	7	+
	89	Bran	96	132	36	454		
	90	Mixed	96	143	47	454		
15	88	Alfalfa	92	115	23	393	7	+
	89	Bran	91	112	21	389		
	90	Mixed	93	126	33	393		
16	88	Alfalfa	80	132	52	438	7	+
	89	Bran	77	115	38	431		
	90	Mixed	78	127	49	433		

# THE CALCIUM AND PHOSPHORUS CONTENT OF SOME ALABAMA VEGETABLES

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The importance of calcium and phosphorus in the diet is well recognized and considerable attention has also been given to the Ca:P ratio. Comparatively few investigations, however, have been made of the constancy of these elements in foods and considerable use has been made of tables, such as Sherman's ('30), for their calculation in given diets. A few typical studies show that from a nutritional standpoint further work along this line is needed.

A study by Frank and Wang ('25) has shown very significant differences in the calcium content of different samples of certain foods. These samples were procured in the market and the causes of the differences are unknown. Gile and Age-ton ('14) showed that while the calcium content of some plants was greatly increased by application of lime to the soil, that of others was not appreciably affected. Storms ('27) found that although New Zealand milk has the same calcium content as that of other countries, the vegetables showed a lower calcium content. The work of Greaves and Hirst ('28) has shown very great differences in the calcium and phosphorus content of certain grains as influenced by variety, soil type and irrigation, and more recently, Greaves and Greaves ('33) have shown that these wheats vary in their nutritive value. Hartwell ('13) found that the percentage of phosphorus in turnips varied with the amount available in the soil, while that of oats and millet was not markedly influenced.

Emmert ('31) and Sewell and Latshaw ('31) found that the phosphorus content of plants was decreased by liming. Cowell ('32) found that the calcium content of the outer leaves of cabbage may be twenty to thirty times that of the inner leaves. Therefore, the extent and significance of these variations should be determined, since diets which would be adequate under certain conditions of plant growth might be deficient under others.

A survey of Alabama shows that the soils of a very considerable part of the state are of very low calcium content and that these soils are not limed in general agricultural practice. Moreover, with a growing tendency to use new kinds of so-called high analysis fertilizers as ammonium phosphate, ammonium nitrate, urea, potassium chloride, etc., the calcium which was formerly added to the soil in fertilizers may be omitted and result in a depletion in the small store of this element. It, therefore, seemed important to determine the calcium content of some vegetable foods grown in these low calcium soils and, because of the relationship of calcium and phosphorus in metabolism, to make a concomitant study of their phosphorus content.

#### MATERIALS AND METHODS

Although it is recognized that there are other important sources of calcium in the diet and that these need investigation, this study was limited to vegetables. Since leafy vegetables are the chief vegetable source of calcium in the diet and since they are much used in the South, most of the study was confined to them. Some analyses, however, were made of other types of vegetables. The field crops were grown on the Alabama Experiment Station at Auburn, with only one rate of fertilization, 1000 pounds per acre of complete fertilizer. The greenhouse plants were grown at Auburn on five different types of soil, all of which, however, were acid and low in calcium. Plants were grown in each soil which received 0, 250, 500, 1000, and 2000 pounds of superphosphate per acre, respectively.

The sample of edible portion was washed in a small amount of distilled water and was freed of wash water by whirling in cheesecloth and spreading on paper towels. It was then weighed in aluminum pans, dried to constant weight at 105°C., ground to a powder in a ball mill or mortar, redried for 2 hours at 105°C. to remove any adsorbed water and stored in Mason jars. Enough dried sample was weighed in a Sillimanite evaporating dish to give 15 to 40 mg. of calcium, ashed in an electric muffle, weighed for ash and dissolved in hydrochloric acid. The calcium was precipitated as the oxalate and titrated with permanganate.

Enough dried sample to give 1.0 to 4.0 mg. phosphorus was weighed in a Sillimanite evaporating dish and 10 cc. 10 per cent calcium acetate solution were added to prevent loss of phosphorus through volatilization. This was evaporated to dryness on a water bath and ashed in an electric muffle. The ash was covered with water and 10 cc. 6*N* HCl, evaporated to dryness on a water bath to precipitate the silica, and then dissolved in water and 4 cc. 1*N* HCl, in order to give the same pH as the standard. It was next filtered into a 200-cc. volumetric flask, made up to volume and the phosphorus in a suitable aliquot, usually 25 or 50 cc., determined by the Fiske and Subbarow ('25) colorimetric method. It was found that the keeping qualities of the reducing agent for this determination were improved by making the solution up to 500 instead of 400 cc. Five cc. were then used in the determination instead of 4 cc., in order to give the same amount of reducing agent.

#### RESULTS

The results summarized in table 1 show a wide variation in the calcium and phosphorus content of a given vegetable. This variation was slightly greater on the basis of dry weight than on the basis of the edible portion which is given in table 1. The calcium content of Alabama vegetables is in general low and the phosphorus content high as compared with Sherman's ('30) tables. Lettuce, mustard, and potatoes, however, are low in phosphorus as well as calcium, while the

TABLE 1

Calcium and phosphorus content (percentage of edible portion) of some Alabama vegetables

VARIETY	NUM- BER SAM- PLES ANAL- YZED	DRY MATTER			ASH			CALCIUM			PHOSPHORUS			Ca:P		
		Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Beans, lima, dried	13	91.5	96.1	94.4	3.42	3.98	3.70	0.064	0.078	0.071	0.368	0.485	0.406	0.161	0.194	0.173
Cabbage, greenhouse, not headed	43	5.92	20.4	13.7	1.14	2.06	1.55	0.204	0.675	0.380	0.009	0.461	0.024	5.12	61.1	19.8
Cabbage, field, headed	2	8.28	10.1		0.636	0.688		0.030	0.046		0.029	0.040		0.755	1.61	
Chard, field	1	13.1				1.65			0.102			0.068			1.50	
Chinese cabbage, greenhouse, non-heading	26	5.63	10.6	7.25	0.936	1.82	1.27	0.083	0.502	0.226	0.012	0.040	0.022	2.56	28.2	11.7
Chinese cabbage, field, non-heading	18	6.16	11.77	8.25	0.925	1.42	1.16	0.086	0.188	0.121	0.038	0.060	0.050	1.45	4.00	2.54
Collards, field	1	13.3				1.29			0.197			0.073			2.71	
Lettuce, greenhouse	34	3.64	14.4	8.45	0.658	1.49	1.03	0.051	0.097	0.071	0.012	0.040	0.023	1.69	6.15	3.37
Mustard, greenhouse	30	5.55	13.5	8.70	0.990	2.32	1.89	0.156	0.431	0.284	0.014	0.050	0.027	3.76	30.0	12.2
New Zealand spinach, field	3	6.33	10.0		1.50	2.00		0.054	0.090		0.031	0.056		1.15	2.19	
Onion, top, field	2	7.14	9.67		0.661	0.895		0.050	0.064		0.025	0.032		2.02	2.03	
Onion, bulb, field	2	9.92	12.4		0.529	0.665		0.049	0.056		0.040	0.044		1.24	1.28	
Peas, cow, dry	14	85.4	88.2	86.9	2.85	3.78	3.21	0.050	0.080	0.064	0.336	0.503	0.442	0.107	0.169	0.145
Pepper, green, field	1	8.86				0.445			0.010			0.030			0.324	
Potato, Irish, field	2	17.9	22.0		0.970	1.19		0.007	0.008		0.042	0.047		0.167	0.173	
Potato, sweet, field	1	32.5				1.17			0.018			0.039			0.471	
Radish tops, greenhouse	8	6.86	9.08		1.18	1.47	1.17	0.170	0.398		0.015	0.034		5.27	23.6	
Tendergreen, green house	22	4.70	8.42	7.10	0.92	1.90	1.32	0.111	0.346	0.217	0.012	0.042	0.025	2.63	19.3	1.00
Tendergreen, field	2	8.86	11.59		1.38	1.39		0.110	0.202		0.056	0.076		1.45	3.60	
Tomato, field	1	5.12				0.467			0.007			0.021			0.341	
Turnip tops, Amer., greenhouse	31	7.05	13.25	9.59	1.27	2.36	1.70	0.212	0.451	0.322	0.016	0.081	0.037	3.23	21.8	10.5
Turnip tops, Amer., field	21	7.58	15.16	10.74	1.16	1.79	1.43	0.180	0.330	0.242	0.046	0.080	0.060	2.39	5.40	4.14
Turnip tops, Jap., greenhouse	20	7.15	10.93	8.53	1.10	1.93	1.47	0.181	0.373	0.274	0.015	0.061	0.031	3.32	20.6	1.01
Turnip tops, Jap., field	9	7.58	14.1	10.20	1.14	1.57	1.31	0.123	0.224	0.172	0.042	0.077	0.060	1.59	4.63	3.12

onion and pepper are high in calcium as compared to Sherman's tables. The amounts found in Alabama vegetables are more comparable to the amounts found in Hawaiian vegetables (Chung and Ripperton, '29) but in general show a slightly higher mineral content. The calcium and phosphorus also show a marked tendency to vary in an inverse manner, thus giving a tremendous variation in the Ca:P ratio.

The greenhouse plants showed a regular increase in phosphorus with increased rates of application of superphosphate.

TABLE 2

*Variation of calcium and phosphorus content with soil and superphosphate added*

VEGETABLE	SOIL	SUPER- PHOSPHATE ADDED, LBS./ACRE	PERCENTAGE OF EDIBLE PORTION				Ca:P
			Dry matter	Ash	Calcium	Phos- phorus	
Cabbage	Cecil	250	15.6	1.75	0.487	0.009	55.0
		2000	19.9	1.49	0.358	0.026	14.0
	Eutaw	250	18.0	1.48	0.700	0.011	61.1
		2000	17.4	1.87	0.675	0.030	22.4
	Hartselle	250	17.2	1.34	0.322	0.017	19.2
		2000	18.6	1.80	0.407	0.043	9.56
Lettuce	Cecil	250	9.60	1.27	0.092	0.015	6.15
		2000	11.6	1.16	0.074	0.027	2.74
	Eutaw	250	9.83	0.993	0.067	0.024	2.83
		2000	14.4	1.018	0.081	0.032	2.52
	Hartselle	250	9.67	1.25	0.066	0.020	3.24
		2000	12.1	0.872	0.070	0.038	1.83

The extent of this variation differed with the vegetable and the soil as is illustrated in table 2. The results are shown for three typical soils with two of the rates of superphosphate for two vegetables, cabbage which showed maximum effect and lettuce which showed the least effect of the vegetables studied. In spite of the fact that superphosphate also contains calcium, the variation in the calcium content with increased rates of superphosphate was small and generally showed a decrease until the highest applications were reached where there was a very slight increase.

Japanese varieties of turnips consistently gave a lower calcium content than American varieties grown under the same conditions. Aside from this, the variation with variety or age was not significant when the values were reduced to a basis of edible portion. Older vegetables, of course, had a higher dry weight and hence in terms of dry weight a lower calcium and phosphorus content.

#### SUMMARY

Analyses were made of a number of Alabama vegetables of which some were grown in the greenhouse on five different soil types with five different rates of superphosphate treatment, and some were grown in the field on one soil type with one fertilizer treatment.

The calcium and phosphorus content of a given vegetable varies widely, usually in opposite directions, so that the Ca : P ratio varies markedly.

Increased rates of superphosphate produce a regular increase in the phosphorus content but a small change in calcium.

#### ACKNOWLEDGMENT

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## LITERATURE CITED

- CHUNG, H. L., AND J. C. RIPPERTON 1929 Utilization and composition of Oriental vegetables in Hawaii. Hawaii Agr. Exp. Sta. Bull., vol. 60, p. 59.
- COWELL, STUART J. 1932 Calcium content of cabbage. Biochem. J., vol. 26, p. 1422.
- EMMERT, E. M. 1931 The effect of soil reaction on the growth of tomatoes and lettuce and on the nitrogen, phosphorus and manganese contents of soil and plant. Ky. Agr. Exp. Sta. Res. Bull., vol. 314, p. 3.
- FISKE, C. H., AND Y. SUBBAROW 1925 The colorimetric determination of phosphorus. J. Biol. Chem., vol. 66, p. 375.
- FRANK, M., AND C. C. WANG 1925 Variation in calcium content of common foods. J. Home Econ., vol. 17, p. 494.
- GILE, P. L., AND C. N. AGETON 1914 The effect of strongly calcareous soils on the growth and composition of certain plants. Porto Rico Exp. Sta. Bull., vol. 16.
- GREAVES, J. E., AND C. T. HIRST 1928 The mineral content of grain. J. Nutrition, vol. 1, p. 293.
- GREAVES, ETHELYN O., AND J. E. GREAVES 1933 Nutritive value of high and low calcium carrying wheat. J. Nutrition, vol. 6, p. 113.
- HARTWELL, BURT L. 1913 The percentage of total phosphorus in flat turnips as influenced by the amount available in the soils. R. I. Exp. Sta. Bull., vol. 154, p. 121.
- SEWELL, M. C., AND W. L. LATSHAW 1931 The effect of lime, superphosphate and potash on reaction of soil and growth and composition of alfalfa. J. Am. Soc. Agron., vol. 23, p. 799.
- SHEERMAN, HENRY C. 1930 Chemistry of food and nutrition. Macmillan.
- STORMS, LILLIAN B. 1927 Calcium content of some New Zealand foods. Trans. and Proc. New Zealand Inst., vol. 58, p. 277.





# VARIATION OF BASAL METABOLIC RATE PER UNIT SURFACE AREA WITH AGE

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## ONE FIGURE

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In a recent article Bruen ('33) gives an excellent summary of the bibliography on this subject. He concludes that the basal metabolic rate during later childhood has been shown in numerous confirmatory investigations to give indications of a definite increase, or an irregularity in its decrease, related to puberty. This variation of the basal metabolism per unit surface area with age during the pubertal period may therefore be analyzed as the mathematical resultant of a fundamental decrease of the basal metabolic rate with age, and a super-imposed cyclic acceleration. This cyclic acceleration of the basal metabolism is not a mere concomitant or effect of the adolescent growth cycle, but represents an independent pubertal metabolic acceleration.

Basal metabolic studies were made on 200 boys whose ages ranged from 9 to 18, the greater number being between 12 and 16 years of age. These boys are all inmates of the New Jersey State Home for Boys in Jamesburg. Our results confirm, in the main, those of DuBois ('16 a, '16 b), Olmstead, Barr and DuBois, ('18), Bierring ('31), Göttche ('26) and Topper and Mulier ('32). Our figures, although they do not show a definite increase, nevertheless do show an irregularity in decrease related to puberty as shown in graph of median trend in chart 1.

The boys, to be tested, were admitted to the institution hospital the evening before. No food was given after the

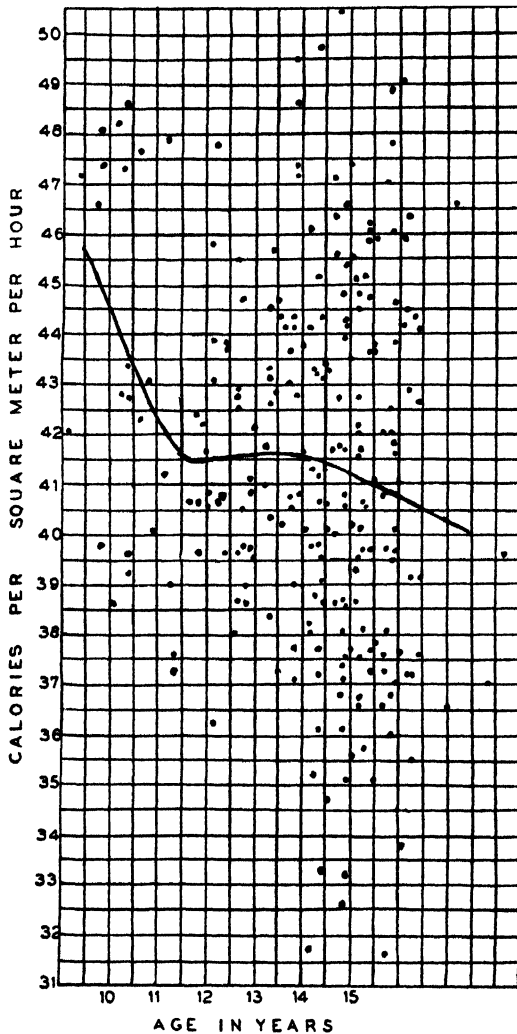


Figure 1

evening meal and nothing to drink after midnight. Successive determinations were made every 24 hours until not more than a 4 per cent difference was obtained. Averages of these check determinations were made. Many cases were excluded be-

cause of inability to obtain a 4 per cent check and because of nervousness. No boys with evident endocrine disturbances were used in the study. All of the tests were done with the Sanborn-Motor-Grafic machine.

Table 1 gives the details of the cases studied. Each age group is separately presented and the averages for each, in

TABLE 1

*Calories per square meter of body surface per hour during puberty (males)*

AGE	TOTAL CASES	APUBERTAL	PUBESCENT	PUBERTAL	AVERAGE (CALORIES PER SQUARE MILLIMETER)	AVERAGE DEVIATION
9-10	6	4	2		45.9	1.9
10-11	7	6	1		43.1	2.5
11-12	8	7	1		40.1	2.4
12-13	22	11	10	1	41.9	1.9
13-14	29	4	17	8	39.7	3.6
14-15	53	5	20	28	36.2	5.1
15-16	58	1	8	49	41.1	3.0
16-17	14		3	11	41.0	3.5
17-18	3			3	40.9	3.8

TABLE 2

*The DuBois normal standard as modified by Boothby and Sandiford (males)  
Calories per square meter*

AGE <sup>1</sup>	BOOTHBY AND SANDIFORD	AUTHORS DATA
10	49.5	45.0
11	48.6	45.0
12	47.8	42.6
13	47.1	41.4
14	46.2	41.5
15	45.3	40.9
16	44.7	41.9

<sup>1</sup> Age—9 years, 6 months to 10 years, 5 months are included in 10-year group, etc.

Calories per square meter per hour, are given. Our figures are lower than those of Aub and DuBois ('17), and Boothby and Sandiford ('29) (table 2). Correlations were made with the data of Dryer ('20) (observed weight formula) and Benedict and Talbot ('21). Table 3 shows at a glance that between the ages of 12 and 15, the range of the majority of

cases, there appears to be a fairly good correlation between Dreyer's and our data. Too few cases were correlated with Talbot's figures to be of any value.

TABLE 3  
*Correlations with Dreyer and Talbot (Calories per hour)*

AGE	DREYER			TALBOT		
	Number of cases	r	P.E.	Number of cases	r	P.E.
9-10	6	0.81	$\pm 0.05$	6	0.81	$\pm 0.05$
10-11	7	0.78	0.06	6	0.65	0.08
11-12	8	0.41	0.12	8	0.32	0.13
12-13	22	0.80	0.05	20	0.51	0.11
13-14	29	0.84	0.05	18	0.77	0.06
14-15	53	0.53	0.07	8	0.63	0.09
15-16	58	0.63	0.06			
16-17	14	0.34	0.13			
17-18	3	0.53	0.11			

### CONCLUSIONS

1. The basal metabolic rate during later childhood (boys) shows an irregularity in its decrease, related to puberty.

2. The average heat production as expressed in Calories per square meter per hour for each age group is given in table 1 which may serve as a set of norms, with the possible exception in the upper and lower age groups.

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## LITERATURE CITED

- AUB, J. C., AND E. F. DUBOIS 1917 The basal metabolism of old men. *Arch. Int. Med.*, vol. 19, p. 823.
- BENEDICT, F. G., AND F. B. TALBOT 1921 Carnegie Institution of Washington no. 302, p. 206.
- BIERRING, E. 1931 The standard metabolism of boys 7 to 18 years, inclusive, Copenhagen, pp. 118-123.
- BOOTHBY, WALTER M., AND I. SANDIFORD 1929 Normal values of basal or standard metabolism. A modification of the DuBois standards. *J. Physiol.*, vol. 90, p. 291.
- BRUEN, C. 1933 Variation of basal metabolic rate per unit surface area with age. *J. Nutrition*, vol. 6, p. 383.
- DREYER, GEORGE 1920 The normal basal metabolism in man. *Lancet*, vol. 11, p. 289.
- DUBOIS, E. F. 1916 a The metabolism of boys 12 and 13 years old compared with the metabolism at other ages. *Arch. Int. Med.*, vol. 17, p. 887.
- 1916 b The respiration calorimeter in clinical medicine. *Am. J. Med. Sci.*, vol. 151, p. 781.
- GÖTTSCHE, O. 1926 Gasstoffwechseluntersuchungen im Kindessalter. Die Pubertätsreaktion. *Monatsschr. f. Kinderheilk.*, Bd. 32, S. 22.
- OLMSTEAD, W. H., D. P. BARR AND E. F. DUBOIS 1918 Metabolism of boys twelve and fourteen years old. *Arch. Int. Med.*, vol. 21, p. 621.
- TOPPER, A., AND H. MULIER 1932 Basal metabolism of normal children. *Am. J. Dis. Child.*, vol. 43, p. 327.



# EXOGENOUS MELITURIA IN MAN <sup>1</sup>

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## THREE FIGURES

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Frequent studies have been made of meliturias induced in man by the ingestion of sufficient carbohydrate to exceed the assimilation limit. Less consideration has been given to certain exogenous factors of the normal diet. Several investigators (Folin and Berglund, '22; Malmros, '28; Höst, '23; West, Lange and Peterson, '32) have demonstrated a slight melituria, lasting 3 to 4 hours, after mixed meals. Höst ('23) found much of this to be unfermentable and to originate chiefly from breads and other baked or toasted carbohydrate foods. Others have noted additional exogenous sources, including dried fruits, commercial sugar syrups, nuts, chocolate, and apples. In studies of these excretory products which we are reporting, we made use of hydrolysis, bromine oxidation, fermentation and the Sumner/Folin-Wu ratio of glucose equivalents.

## METHODS

The analytical, fermentation and bromine oxidation procedures have been previously described (Everett, Shoemaker and Sheppard, '27; Everett, Edwards and Sheppard, '34; Everett and Sheppard, '28; Everett and Edwards, '33;

<sup>1</sup> A preliminary report of these experiments appeared in the biochemical abstracts of the eighty-fourth meeting of the American Chemical Society, August, 1932.



Everett, '30). Everett, Shoemaker and Sheppard ('27) reported that 0.28 N sulfuric acid was sufficient to give maximal values for hydrolyzable sugar in normal urine, but the buffering effect of urine makes it necessary to use 2.5 N acid in hydrolyzing added lactose, maltose and dextrin. For this purpose we added 1 cc. of 20 N sulfuric acid solution to 7 cc. of urine filtrate in a total sugar tube and heated the mixture 2.5 hours upon the boiling bath. After cooling, 2 cc. of 10 N silicate-free potassium hydroxide solution were added. In control experiments, with sugars added to urine, hydrolysis by the more concentrated acid was an improvement, although not entirely satisfactory because of the destruction of some free sugar, especially ketoses. Hydrolysis with N hydrochloric acid produced even greater destruction as estimated by Sumner's method. Deuel, Waddell and Mandel ('26) have reported similar destruction of lower anhydro sugars by hydrochloric acid. Since hydrolysis of urine with a single concentration of acid was obviously deficient in one respect or another, we used both 0.28 and 2.5 N sulfuric acid to secure all possible information. Even with this provision, hydrolyzable sugar determinations have only a semi-quantitative significance.

The experimental subjects were normal adults of both sexes. Night urines were discarded and 1 to 2 hour basal samples collected. The weighed foods were then given, together with 500 cc. portions of water, and urine again collected for the next 3 hours. In most experiments we limited our study to this early excretory period, because the greatest exogenous melituria occurs during this interval (Malmros, '28; Page, '22).

The results, given in figures 1 to 3, are averages of 3 to 10 experiments upon different individuals. Excretions referable to the ingested foods were calculated by subtracting the basal values from those of the corresponding post-prandial periods.

*Bread and dextrins*

We first investigated the effects of feeding bread. One hundred and fifty gram portions of whole wheat and rye breads caused the excretion of approximately 5 mg. of reducing sugar per hour, together with traces of hydrolyzable sugar. The excreted material could be regarded as dextrins or simpler anhydro sugars, according to one's view of the reactions occurring in baking. It is well known that heating starch results in the formation of dextrins, and that those in the crust are subsequently caramelized. Kalning and Schleimer ('19) believe caramelization to be the principal toasting process. Unfortunately, the words 'dextrinization' and 'caramelization' are used to designate a variety of chemical reactions.

Before attempting further analysis of the excretory products from toast, it is desirable to consider briefly the results of feeding larger amounts of dextrins and anhydro sugars than are possible through the medium of bread. Starch and glycogen may be ingested by man in large quantities without melituria (Allen, '13; Cammidge, '27). Early experimenters believed that dextrins acted similarly, but Folin and Berglund, ('22) found 100 to 200 mg. of hydrolyzable sugar excreted hourly after 200 to 300 gm. of pure commercial dextrin. Unable to confirm this excretion by feeding somewhat smaller amounts of dextrin prepared by amylase, they attributed the excretion to unusable carbohydrate impurities in commercial dextrins. A better explanation is afforded by assuming that they exceeded the assimilation limit of their subject in the first instance, because recent investigators (von Hoesslin and Pringsheim, '23; Lustig and Landau, '32; West, Lange and Peterson, '32) have shown that 100 gm. portions of dextrin cause no melituria—a fact which we have verified by feeding Merck's C. P. dextrin. From these considerations it is obvious that the excretory products from bread and toasted foods are not to be regarded as dextrins.

Maltose is also completely utilized by man (Voit, 1897; Folin and Berglund, '22; Greenwald, Gross and Samet, '24) and

may be dismissed from considerations of exogenous melituria. We have fed 125 gm. of this sugar to normal adults without melituria. In the systemic blood of these subjects the reducing sugar increased 30 mg. per cent by the Folin-Wu method, but the hydrolyzable sugar only 3 mg. per cent, signifying that only traces of maltose arrive at the kidneys.

### *Toast and anhydro sugars*

Nonnenbruch ('25), Kerb and Kerb-Etzdorf ('24) and Grafe and von Schroeder ('24) have shown that man excretes small amounts (usually less than 10 per cent) of ingested lower anhydro sugars. These carbohydrates appear to be absorbed unchanged (Grafe and von Schroeder, '24); cause little increase in blood sugar (Kerb, '24; Peters, '30); and produce no glycosuria in normal or diabetic animals (Deuel, Waddell and Mandel, '26; Grafe, '14; Umber, '15; von Noorden, '17; Reimer, '20). The intermediate anhydro sugars, tetraglucosan and caramel, are partly excreted by dogs, but not by man (Peters, '30; Greenwald, Gross and McGuire, '27). Large amounts of caramel (125 gm.) cause an excretion of considerable unfermentable reducing sugar in man, together with much hydrolyzable sugar (West, Lange and Peterson, '32). After hydrolysis, the latter is fermentable.

According to Grafe ('24), the higher anhydrides, obtained by roasting cereal polysaccharides, are utilized without much excretion, but others (West, Lange and Peterson, '32; Greenwald, Gross and Samet, '24) have found that a highly toasted cereal,<sup>2</sup> causes the excretion of unfermentable sugar (approximately 400 mg. from 100 gm. of the cereal). We have fed 150 gm. portions of this cereal with the results shown in figure 1. There was a marked excretion of reducing material which had a Sumner/Folin-Wu ratio of 1.7. Approximately 70 per cent of this material was non-fermentable, and 60 per cent was not oxidized by bromine in 48 hours. Note that the Sumner/Folin-Wu ratio of the extractable reducing sugar of the cereal is 2.2. One hundred gram portions of ordinary toasted

<sup>2</sup> Grape Nuts.

bread led to the excretion of smaller amounts of reducing material with a ratio near unity (fig. 1). In neither case was hydrolyzable sugar excreted.

These excretory products were not dextrans or their non-fermentable oligosaccharide impurities (Everett and Edwards,

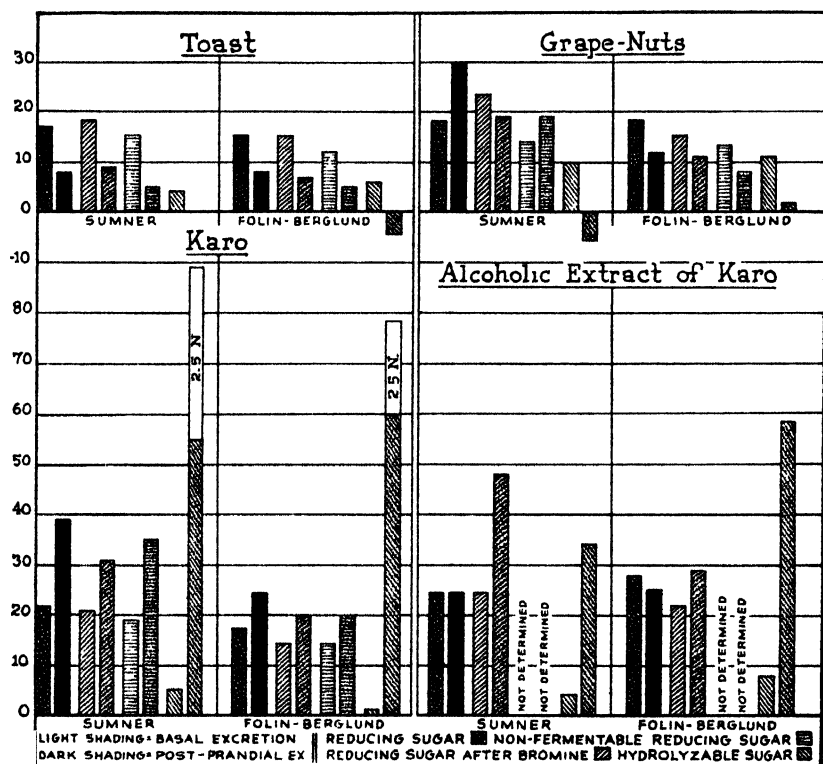


Figure 1

'33) because: hydrolyzable sugar was absent; similar amounts of dextrin caused no melituria; the excretory products were not precipitated by alkaline mercury, while added dextrin was.<sup>3</sup> They resembled lower anhydro sugars in their destruc-

\* A curious phenomenon results when dextrin is added to normal urine. More reducing sugar appears than can be accounted for by the original oligosaccharide impurities of the added dextrin. The Sumner/Folin-Wu ratio is 2 for the material in urine, but 4.5 for that in the dextrin itself. Previous heating of urine at 100° C. for 5 minutes decreases this effect considerably.

tion by acid, even hydrolysis with 0.28 N acid causing negative hydrolyzable sugar values. In this respect, and also in their greater resistance to bromine oxidation, they differed from caramel. The lack of hydrolyzable sugar differentiated them from ordinary anhydro sugars. The products formed by toasting cereal polysaccharides are not limited to dextrans and ordinary anhydro sugars. At these high temperatures, more deepseated dehydration occurs, and the presence of oxygen may result in the formation of anhydro sugar acids or their lactones. We have searched the literature for an indication of the extent of dehydration and oxidation during the process of starch toasting and have been unable to find any consideration of this topic. We have undertaken a study of the transformations of starch at baking temperatures, but details of these experiments must be given later. It may be stated, however, that lactone formation has been detected.

### *Glucose syrups*

Wang and Felsher ('24) observed melituria in man following commercial granular glucose per os. (See also Blatherwick, Bell, Hill and Long, '25.) They extracted the melituristic fraction from commercial glucose by means of alcohol and found that the extract was ineffective when fed alone, but caused melituria when given with glucose. This result is difficult to harmonize with accepted physiologic mechanisms, and we are inclined to attribute the observed phenomena to a difference in behavior of the excreted carbohydrate with the several analytical reagents employed.<sup>4</sup> This glucose impurity should be present in greater concentrations in the commercial syrups from which the granular glucose is crystallized. With this in mind, we made careful studies of the common syrups<sup>5</sup> whose composition is given in table 1.

<sup>4</sup> Dr. C. C. Wang, in a personal communication, informed us that the Folin-Wu method was used for blood sugar and the Benedict-Osterberg method for urine sugar in their experiments.

<sup>5</sup> Karo, blue, red, and orange label.

The results of syrup feeding, as given in figure 1, are averages of many experiments in which we administered 2.7 gm. of blue label syrup per kilogram of body weight. Similar experiments with red and orange label syrups are not detailed here,

TABLE 1  
*A. Authors' analyses of foods (sugars as per cent glucose)*

FOOD	METHOD	REDUCING SUGAR				HYDROLYZABLE SUGAR	
		Original	Non-fermentable	After bromine	S/F-W ratio	0.28 N acid	2.5 N acid
Caramel	Sumner	15	14	7		25	40
	Folin-Wu	12	11	5	1.25	23	37
Dextrin <sup>1</sup>	Sumner	26	22	32		59(78) <sup>1</sup>	70(81) <sup>1</sup>
	Folin-Wu	6	5	7	4.3	67(72) <sup>1</sup>	81(84) <sup>1</sup>
Karo (blue label)	Sumner	47	28			22	20
	Folin-Wu	26	10		1.8	32	34
Karo (alcoholic extract)	Sumner	58				16	
	Folin-Wu	50			1.15	20	
Karo (alcoholic precipitate)	Sumner	36				29	
	Folin-Wu	11			3.3	43	
Hydrol	Sumner	56	17	6		13	21 <sup>2</sup>
	Folin-Wu	49	13	5	1.15	19	25 <sup>2</sup>
Cane molasses	Sumner	20	4			24	19
	Folin-Wu	19	4		1.05	23	16

<sup>1</sup> Parenthetical values corrected for destruction of oligosaccharide impurities.

<sup>2</sup> Merck's C.P., containing 10 per cent moisture.

<sup>3</sup> Difficultly split by bromine.

From 1000 gm. of blue label Karo obtained 145 gm. of alcoholic extract concentrate and 415 gm. of alcoholic precipitate.

*B. Manufacturer's analysis of Karo syrup (per cent)*

	BLUE LABEL	RED LABEL	ORANGE LABEL	HYDROL
Moisture	25.3	25.0	25.4	19.5
Dextrins	37.1	35.5	35.6	21.6
Maltose	22.2	21.8	22.5	
Dextrose	7.5	7.3	7.5	56.4
Sucrose	4.8	9.7 <sup>1</sup>	9.0	
Invert sugar	2.3			

<sup>1</sup> Calculated from formula.

but yielded almost identical results. The considerable excretion of reducing sugar which followed the ingestion of blue label syrup reached its peak during the second post-prandial hour. In addition there appeared huge amounts of rather difficultly hydrolyzable sugar, which remained unnoticed by Wang and Felsher. This material could be neither dextrin nor dextrin oligosaccharide for reasons already discussed in the preceding section of this report. Fermentation and bromine oxidation indicated that neither maltose nor glucose was excreted.

We prepared an alcoholic extract of the blue label syrup by repeated extraction with 2 volumes of 95 per cent alcohol (table 1). The combined extracts were evaporated to a syrup upon the water bath. Corresponding portions of this syrup (0.4 gms. per kilogram of body weight) and of the alcohol-insoluble fraction (1.1 gms. per kilogram of body weight) were given to individuals who had previously taken the blue label syrup. These experiments indicated clearly that the alcoholic extract (fig. 1) contained practically all of the melituric factor while the precipitate (fig. 2) had none. The hydrolyzable sugar of the alcoholic extract was less than one-fifth of that in the original syrup, yet it was the sole source of the hydrolyzable excretory product. The latter, during the 3 hours under investigation, represented less than 1 per cent of the original hydrolyzable sugar.

These results, differing considerably from those reported by Wang and Felsher, demonstrated that the excretory material from glucose syrups was a reducing saccharide of comparatively low molecular weight. The same oligosaccharide was present in the non-fermentable fraction of hydrol syrup (the commercial designation for the mother liquor from commercial glucose crystals). This non-fermentable fraction has been variously named 'isomaltose', 'gallisin', etc. Berlin ('26) identified 18 per cent of it as gentiobiose. However, the behavior of the excretory product with bromine in our experiments indicated that it was not gentiobiose, but rather an unidentified saccharide of the gallisin

fraction. This excretory product was obviously different from that due to toasted foods. It differed also from the hydrolyzable sugar of normal urine by being split with greater difficulty.

We wish to acknowledge several courtesies of the Corn Products Refining Company and of Dr. Henry Berlin in connection with this part of our investigation.

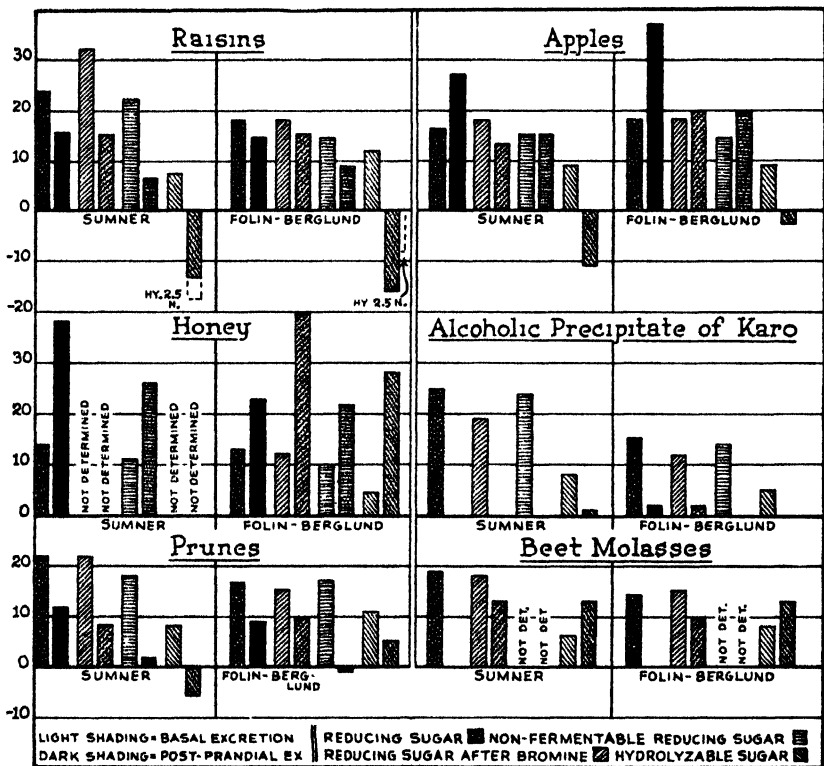


Figure 2

### Miscellaneous foods

Experimental findings with other sugar syrups are given in figures 2 and 3. Three grams of honey per kilogram of body weight resulted in the excretion of considerable reducing sugar which was largely non-fermentable and not destroyed



by bromine. In addition, there was an excretion of hydrolyzable sugar which was easily split by bromine (as determined by hydrolysis of the bromine-oxidized filtrates). This material resembled sucrose in several respects, although one would scarcely expect the 4 gm. of sucrose taken by these subjects in the honey to exceed their assimilation limits.

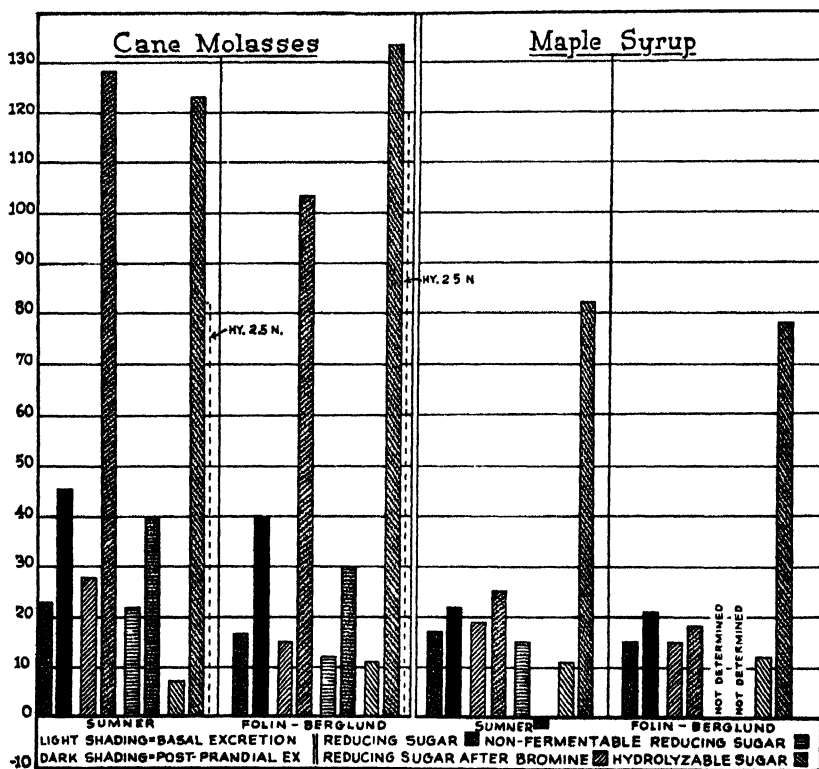


Figure 3

Harding and Selby ('31) found the reducing material excreted after honey to be partly fermentable. West, Lange and Peterson ('32) found it non-fermentable.

Cane molasses, in portions of 2.7 gm. per kilogram of body weight, resulted in the excretion of non-fermentable and bromine-resisting reducing material, together with large amounts of hydrolyzable sugar, which was easily split by 0.28 N acid

and by bromine. Hydrolysis with 2.5 N acid partially destroyed the liberated sugar, which was stable to bromine. The excretory product was evidently a ketose saccharide, but neither sucrose nor raffinose (Everett, Edwards and Sheppard, '34). The molasses fed contained only 50 gm. of sucrose, which should cause no melituria. The fermentable reducing sugar, also noted by Harding and Selby ('31), is a relatively small fraction of the excreted material.

We also fed beet molasses, furnished by the Great Western Refining Company, but only in quantities of 0.35 gm. per kilogram of body weight, because of the high content of salts and extractives. The results were similar to those with cane molasses.

Maple syrup, in portions of 3 gm. per kilogram of body weight, caused the appearance of approximately 20 mg. per hour of fermentable reducing sugar. In one experiment considerable amounts of hydrolyzable sugar were also excreted. This material resembled sucrose.

Five hundred grams of apples caused a considerable excretion of reducing sugar which had a Sumner/Folin-Wu ratio of 0.75. Approximately 50 per cent of this material was fermentable and destroyed by bromine. In other experiments equivalent amounts of cooked apple sauce caused no melituria. It may also be recalled that Folin and Berglund ('22) found a considerable melituria after cider. In our experiments 150 gm. of raisins and 450 gm. of prunes caused similar excretions. With fruits the hydrolyzable sugar of urine tended to be negative, indicating the excretion of rather labile reducing substances. Four hundred grams of bananas and 1000 gm. of peeled oranges caused no melituria, but Harding and Selby ('31) claimed that some fermentable reducing sugar appeared after 500 cc. of orange juice.

Other substances which we found to have no effect were 175 gm. of Fleischmann's yeast, 250 gm. of broiled liver, 500 cc. of cereal beverage and 35 gm. of malt extract.

*The hydrolyzable sugar of normal urine*

Everett, Edwards and Sheppard ('34) have summarized the properties of normal urine sugars. In considering the hydrolyzable fraction, it may be recalled that parenteral injections of soluble starch (Voit, 1897; Bernard, 1877; von Leube, 1897; Mendel and Mitchell, '05; Verzar, '11), of amylo- and erthrodextrin (Allen, '13; Voit, 1897; Mendel and Mitchell, '05; Mayer, '03), and of glycogen (Allen, '13; Mendel and Mitchell, '05; Mayer, '03; Boehm and Hoffman, 1877; Pavy, 1899) cause the appearance in urine of considerable amounts of hydrolyzable sugar, which has been called achroö-dextrin. Allen ('13) suggested that the kidney itself was the source of this material, and one might postulate a similar origin for the hydrolyzable sugar of normal urine, which has also been called an achroödextrin (Cammidge, '27). The present investigation has demonstrated that the hydrolyzable sugar of normal urine is easily hydrolyzed and that it is not precipitated by alkaline mercuric reagents. Hence it is not a dextrin. The major portion of this material differs from conjugated glycuronate and also from the hydrolyzable sugar of tungstic acid blood filtrates (Everett and Sheppard, '28). The latter is more difficult to hydrolyze and part of it is precipitated as uranyl nucleotide.<sup>6</sup>

Our metabolic data do not indicate that the carbohydrate foods administered in these experiments stimulated normal sugar excretion. Following toasted foods, syrups and fruits the excreted reducing material resembled the normal uroketose, but the hydrolyzable sugar was either absent or very different; after molasses, the proportion of free to hydrolyzable sugar was very small; the excreted reducing sugar following maple syrup was fermentable, and that from fruits more fermentable than normal sugars; after honey, the Sumner/Folin-Wu ratio for the reducing sugar was high. These metabolites are not identical with the sugar of normal night urine. The reducing uroketose and the hydrolyzable sugar

\* Unpublished experiments performed in this laboratory by Dr. Wayne M. Hull.

(perhaps two expressions for one substance) remain unexplained metabolic phenomena, whose origin is to be sought in intermediate metabolism. They are not directly traceable to impurities in dietary components and we concur in the opinion of Greenwald, Gross and McGuire ('27) that the excretion of foreign sugars is responsible for only a small fraction of normal urine sugars.

#### SUMMARY

The authors have investigated the nature of the carbohydrate excretory products of human urine resulting from the ingestion of breads, toasted foods, sugar syrups, fruits, etc. Characteristic metabolites arise from each of these. The substance excreted after toasted cereals is neither a dextrin nor a simple anhydro sugar, but rather an unknown product of starch toasting which is being further investigated. After the ingestion of glucose syrups, there appears an unknown, non-fermentable reducing saccharide of low molecular weight. This material is to be found in the 'isomaltose' fraction of glucose syrups.

Certain phases of the metabolism of dextrins and anhydro sugars have been considered. These substances do not appear to have any relation to the hydrolyzable sugar of normal urine. Methods for determining the latter have been considered.

The origin of the major portion of the uroketose and the hydrolyzable sugar of normal night urine is not to be sought in the articles of diet investigated, but breads and fruits are ordinarily responsible for a small portion of the day sugars.

#### LITERATURE CITED

- ALLEN, F. M. 1913 Glycosuria and diabetes. W. M. Leonard, Boston.  
BERLIN, H. 1926 Occurrence of gentiobiose in the products of the commercial hydrolysis of corn starch. J. Am. Chem. Soc., vol. 48, p. 2627.  
BERNARD, C. 1877 Le Diabete. Paris.  
BLATHERWICK, N. R., M. BELL, E. HILL, AND M. L. LONG 1925 The excretion of normal urine sugar. J. Biol. Chem., vol. 66, p. 801.  
BOEHM, R., AND F. A. HOFFMANN 1877 Verhalten des Glykogens nach injektion in den Blut Kreislauf. Archiv. f. exp. Pathol. u. Pharmacol., Bd. 7, S. 489.  
CAMMIDGE, P. J. 1927 Dextrinuria. Lancet, vol. 2, p. 1386.

- DEUEL, H. J., S. S. WADDELL, AND J. A. MANDEL 1926 Physiological behavior of glucosan. *J. Biol. Chem.*, vol. 68, p. 801.
- EVERETT, M. R. 1930 The reducing action of glutathione. *J. Biol. Chem.*, vol. 87, p. 761.
- EVERETT, M. R., AND B. G. EDWARDS 1933 Reduction by oligosaccharides. *J. Biol. Chem.*, vol. 100, p. 43.
- EVERETT, M. R., B. G. EDWARDS, AND F. SHEPPARD 1934 The behavior of carbohydrates in bromine water and the ketose of normal urine. *J. Biol. Chem.*, vol. 104, p. 11.
- EVERETT, M. R., AND F. SHEPPARD 1928 The hydrolyzable sugar of blood. *J. Biol. Chem.*, vol. 80, p. 255.
- EVERETT, M. R., H. A. SHOEMAKER, AND F. SHEPPARD 1927 Total sugar of blood and urine. *J. Biol. Chem.*, vol. 74, p. 739.
- FOLIN, O., AND H. BERGLUND 1922 Some new observations and interpretations with reference to transportation, retention and excretion of carbohydrates. *J. Biol. Chem.*, vol. 51, p. 213.
- GRAFE, E. 1914 Über die Wirkung des Karamels in normalen u. diabetischen organismus. *Deutsch. Archiv. f. klin. Med.*, Bd. 43, S. 402.
- 1924 Das Karamel in der Diabetes Therapie. *Ergeb. ges. Med.*, Bd. 5, S. 449.
- GRAFE, E., AND E. VON SCHROEDER 1924 Studien mit Lävoglucosan. *Deutsch. Archiv. f. klin. Med.*, Bd. 144, S. 156.
- GREENWALD, I., J. GROSS, AND G. MCGUIRE 1927 Observations on the nature of the sugar of normal urine. *J. Biol. Chem.*, vol. 75, p. 491.
- GREENWALD, I., J. GROSS, AND J. SAMET 1924 The sugar excretion upon various diets and the influence of diet upon glucose tolerance, with some remarks on the nature of the action of insulin. *J. Biol. Chem.*, vol. 62, p. 401.
- HARDING, V. J., AND D. L. SELBY 1931 Fermentable sugar in normal urine. *Biochem. J.*, vol. 25, p. 1815.
- VON HOESSLIN, H., AND H. PRINGSHEIM 1923 Glykogenbildung u. tierische Verbrennung. *Zeit. f. physiol. chem.*, Bd. 131, S. 168.
- HÖST, H. F. 1923 The urine sugar and its relation to the blood sugar. *J. Metabol. Res.*, vol. 4, p. 315.
- KALNING, H., AND A. SCHLEIMER 1919 Dextrinbestimmungen im Mehl, Teig u. Gebäck. *Zeit. f. ges. Getreidewesen*, Bd. 11, S. 112.
- KERB, J. 1924 Einige Untersuchungen mit Tetraglucosan. *Zeit. f. ges. exp. Med.*, Bd. 43, S. 402.
- KERB, J., AND E. KERB-ETZDORF 1924 Das physiologische Verhalten der Glucosane. *Biochem. Zeit.*, Bd. 151, S. 435.
- VON LEUBE, C. 1903 Extrabuccale Ernährung bei Menschen. *Deutsche Klinik*.
- LUSTIG, B., AND L. LANDAU 1932 Über den Gehalt des normalen Harnes an Kohlenhydraten u. deren Beziehungen zur Nahrung, *Biochem. Zeit.*, Bd. 245, S. 174.
- MALMEOS, H. 1928 A study of glycosuria. *Acta Med. Skan.*, supplement 28.
- MAYER, P. 1903 Verhalten von Dextrin u. Glykogen im Tierkörper. *Fortschritte d. Med.*, Bd. 21, S. 417.

- MENDEL, L. B., AND P. H. MITCHELL 1905 On the utilization of various carbohydrates without intervention of the alimentary digestive processes. *Am. J. Physiol.*, vol. 14, p. 239.
- NONNENBRUCH, W. 1925 Die Wirkung von Anhydrozucker in normalen Personen und Diabetikern. *Zeit. f. ges. exp. Med.*, Bd. 48, S. 232.
- VON NOORDEN, C., AND S. ISAAC 1928 Die Zuckerkrankheit und ihre Behandlung. 8th ed. Berlin.
- PAGE, I. H. 1922 Normal urine sugar curve in normal individuals, borderline diabetics and severe diabetics under insulin treatment. *J. Lab. and Clin. Med.*, vol. 8, p. 631.
- PAVY, G. W. 1899 Subcutaneous injections of various carbohydrates. *J. Physiol.*, vol. 24, p. 479.
- PETERS, F. 1930 Über die Ausnutzbarkeit der Anhydrozucker im menschlichen u. tierischen Organismus. *Deutsch. Archiv. f. klin. Med.*, Bd. 168, S. 301.
- REIMER, G. 1920 Karamel in der Behandlung der Diabetiker. *Deutsch. Archiv. f. klin. Med.*, Bd. 132, S. 219.
- UMBER, F. 1915 Karamose für Diabetiker und Kinder. *Deutsch. med. Wochschr.*, Bd. 41, S. 181.
- VERZAR, F. 1911 Parenteraler Stärkstoffwechsel. *Biochem. Zeit.*, Bd. 34, S. 66.
- VOIT, F. 1897 Verhalten verschiedenen Zuckerarten. *Deutsch. Archiv. f. klin. Med.*, Bd. 58, S. 523.
- WANG, C. C., AND A. R. FELSHER 1924 The effect of alcoholic extract of commercial granular glucose on urinary reducing substances. *J. Biol. Chem.*, vol. 61, p. 659.
- WEST, E. S., A. C. LANGE, AND V. L. PETERSON 1932 Factors affecting the excretion of fermentable and non-fermentable sugars in urine. *Biochem. J.*, vol. 26, p. 1728.



## VITAMIN STUDIES

### XIX. THE ASSIMILATION OF CAROTENE AND VITAMIN A IN THE PRESENCE OF MINERAL OIL

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#### SEVEN CHARTS

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In a previous paper from this laboratory (Dutcher, Ely and Honeywell, '27), experiments were described which indicated that rats are unable to utilize vitamin A in butter fat when the latter is dissolved in mineral oil. At that time we concluded that "mineral oil may act as a solvent for vitamin A thereby depleting the ingested foods of their supply of this vitamin." Our findings were in general agreement with those of Burrows and Farr ('27) who postulated that unabsorbed mineral oil might act as a solvent for vitamin A in the food and in the mucosa of the digestive tract.

Subsequent experiments in our laboratory indicated quite clearly that the above generalization was not applicable to vitamin A in cod liver oil, since the latter (in mineral oil) seemed to be utilized more efficiently than butter fat when fed at corresponding levels and under similar conditions. These results substantiated the observations of Moness and Christianson ('29), who described experiments in which a concentrate prepared from cod liver oil was dissolved in olive oil and in mineral oil, respectively. These authors concluded



that the utilization of vitamin A from cod liver oil is not appreciably affected by the presence of mineral oil.

Rowntree ('31), working with cod liver oil, concluded that the utilization of vitamin A is lowered by mineral oil only when the cod liver oil is fed at relatively low levels. She expressed the belief that mineral oil can be used with impunity if the vitamin A content of the diet 'is adequate.' Since our interest in the problem relates solely to the question of the use of mineral oil as a vehicle or solvent for vitamin A in the biological assay of food materials, we have purposely confined our vitamin A intakes to those levels which would be considered relatively low.

Jackson ('31) repeated our experiments, using minimal doses of butter fat, and concluded that mineral oil tends to prevent the utilization of vitamin A when butter fat and mineral oil are mixed prior to ingestion, but that practically no detrimental effects can be noted when butter fat and mineral oil are administered separately.

The relatively recent discovery (Moore, '29, '30) that the pigment carotene is the precursor or parent substance of vitamin A seemed to offer a possible explanation for the apparently diverse results obtained with pigmented butter fat and non-pigmented cod liver oil.

The experiments described in the present paper were undertaken to determine the effect of mineral oil on the utilization of, a) purified carotene, b) a pigment-free cod liver oil, and, c) a vitamin A concentrate prepared therefrom, and to ascertain, if possible, the mechanism whereby mineral oil lowers the vitamin potency of butter fat.

#### EXPERIMENTAL

The rats used in this study were placed on experiment at 21 days of age, at which time they averaged about 40 gm. in weight. All animals were maintained in individual metal cages throughout the entire experimental period. The cages were equipped with false bottoms consisting of heavy wire screening containing two meshes to the inch.

The vitamin A-free ration consisted (in parts per 100) of casein 18, agar 2, dextrin 77, and McCollum and Simmond's no. 185 ('17) salt mixture 3. Vitamins B and G were furnished by daily allotments (0.6 gm.) of yeast which were fed separately from the basal ration to insure a constant and ample supply (Honeywell, Dutcher and Ely, '31) of these vitamins. The basal ration was irradiated with a carbon arc lamp at a distance of 18 inches for 20 minutes (Dutcher and Kruger, '26) to insure sufficient vitamin D for the experimental period.

All animals were depleted of their vitamin A reserves by feeding the basal diet until they showed evidence of vitamin A deficiency, i.e., by the appearance of incipient xerophthalmia or by loss of body weight. At this point the materials to be assayed were fed in measured amounts separate from the basal ration and the curative period was continued for 5 weeks. Evidence for the assimilation of ~~sufficient~~ amounts of carotene or vitamin was considered satisfactory when xerophthalmic symptoms disappeared and when the rate of growth averaged not less than 3 gm. per week during the 5-week curative period. Each experimental group consisted of at least five rats with the exception of a few groups which contained but three or four individuals per group, owing to shortage of animals.

The vitamin A concentrate used in this study was prepared by slight modifications of the method of Drummond, Channon and Coward ('25) which consisted essentially of saponification of 900 gm. of cod liver oil with alcoholic potassium hydroxide, extraction of the unsaponifiable fraction with ether, and evaporation of the ether in an atmosphere of carbon dioxide. Cholesterol was removed from the yellow semi-solid mass by freezing out in methyl alcohol, after which the alcoholic solution of vitamin A was evaporated in an atmosphere of carbon dioxide to a light yellow semi-solid fraction. This fraction, which weighed 0.5 gm., was dissolved in corn oil and made up to a volume of 500 ml. One cubic milliliter of this solution was again dissolved in corn oil and made up to a volume of

250 ml. One-half ml. of this solution was found to contain one Sherman unit of vitamin A, which is equivalent to 500,000 Sherman units per gram of concentrate.

The crystalline carotene used in this study was also dissolved in corn oil and all levels of carotene and vitamin A concentrate were adjusted so that all dosages were administered in  $\frac{1}{2}$  ml. of corn oil (daily) in order that variations might be eliminated due to fluctuations in daily intakes of corn oil.

#### FIRST SERIES OF EXPERIMENTS

In the first series we repeated our former experiments with butter fat, which was fed at two levels, viz., 20 and 40 mg.

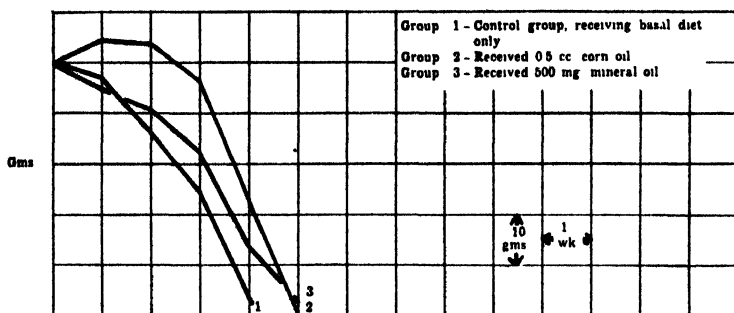


Chart 1

daily. In order to prove that the presence of corn oil had no detrimental effect, two control groups were fed vitamin A-free diets. One of these (group 2) received 0.5 ml. of corn oil (daily) during the curative period. A third control group received the basal diet with additions of 500 mg. of mineral oil (daily) throughout the curative period. The results obtained are summarized in chart 1.

In order to eliminate the possibility of loss of carotene or vitamin A by possible oxidative changes due to the presence of oxidants, two of the butter fat diets (groups 6 and 9) were protected by the anti-oxidant hydroquinone. The results of these experiments are summarized in chart 2.





Groups 10 to 20 inclusive were fed the basal ration supplemented with carotene during the curative period to establish the potency of the carotene employed. It will be noted (chart 3) that 2.5  $\gamma$  of carotene (daily) were equivalent to one Sherman unit.

Chart 4 summarizes the data obtained when various levels of carotene were fed during the curative period in the presence

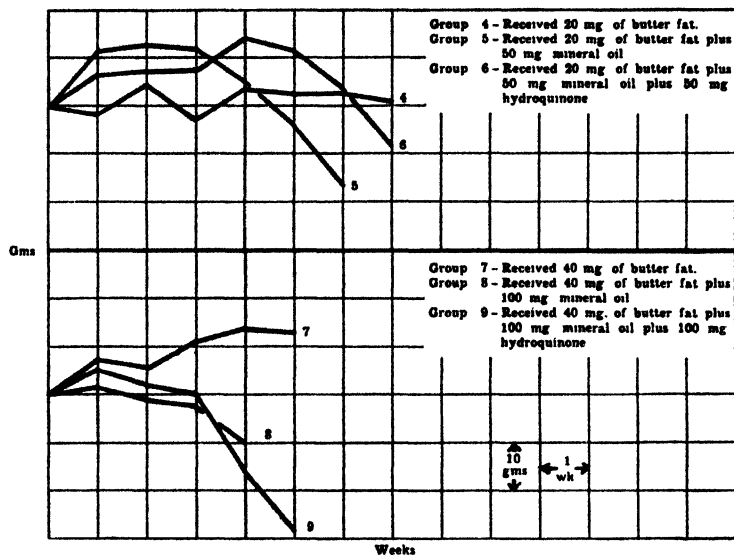


Chart 2

and absence of varying amounts of mineral oil and in the presence and absence of hydroquinone.

Groups 23 to 28 inclusive received varying daily allotments of cod liver oil in the presence and absence of varying amounts of mineral oil. These results are summarized in chart 5.

Chart 6 shows the results obtained when  $2 \times 10^{-6}$  mg. of cod liver oil concentrate were fed in the presence and absence of mineral oil.

### *Discussion of results obtained in first series of experiments*

The results obtained with butter fat dissolved in mineral oil are in general agreement with those previously described

(Dutcher, et al., '27; Jackson, '31). It is clear that mineral oil, in some manner, prevents the utilization of carotene or vitamin A or both. The fact that hydroquinone (chart 2) did not prevent this deleterious effect would seem to support the postulation that the loss of vitamin A activity is not due to the presence of a pro-oxidant in mineral oil.

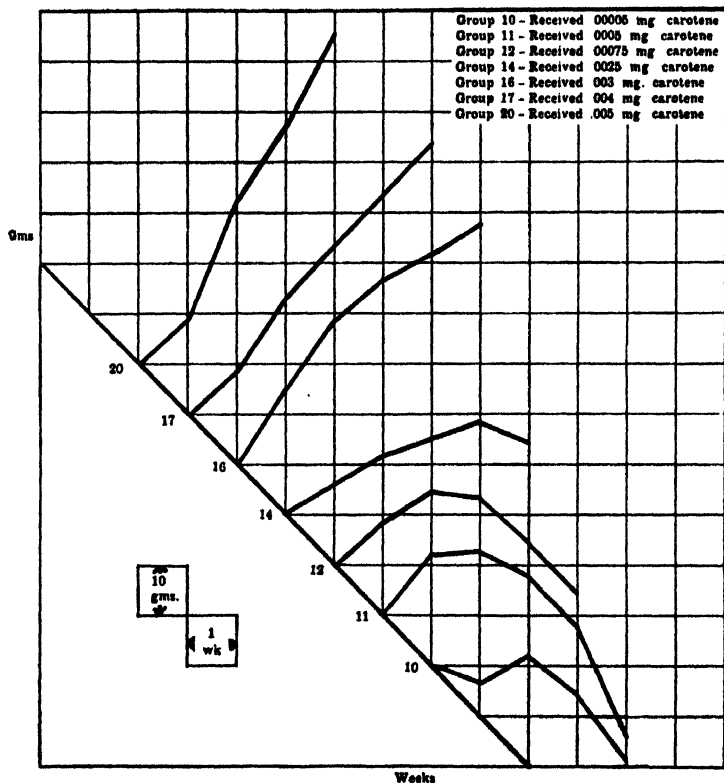


Chart 3

When carotene was fed as the sole source of vitamin A activity, the results were even more convincing (chart 4). When carotene was fed in daily doses of 2.5  $\gamma$ , as little as 6.25  $\gamma$  of mineral oil caused marked retardation of growth (groups 14 and 15). When carotene was fed in excess of the Sherman unit requirement (groups 17, 18 and 19), better

growth responses were obtained, but the effect of the small amount of mineral oil was still quite marked. It will be noted, also, that the presence of 100 mg. of hydroquinone did not prevent the deleterious effect of the mineral oil. When the dosage of carotene was increased to 5  $\gamma$ , growth response was excellent, except where mineral oil was added. When an excessive amount of mineral oil (2 ml.) was fed daily in

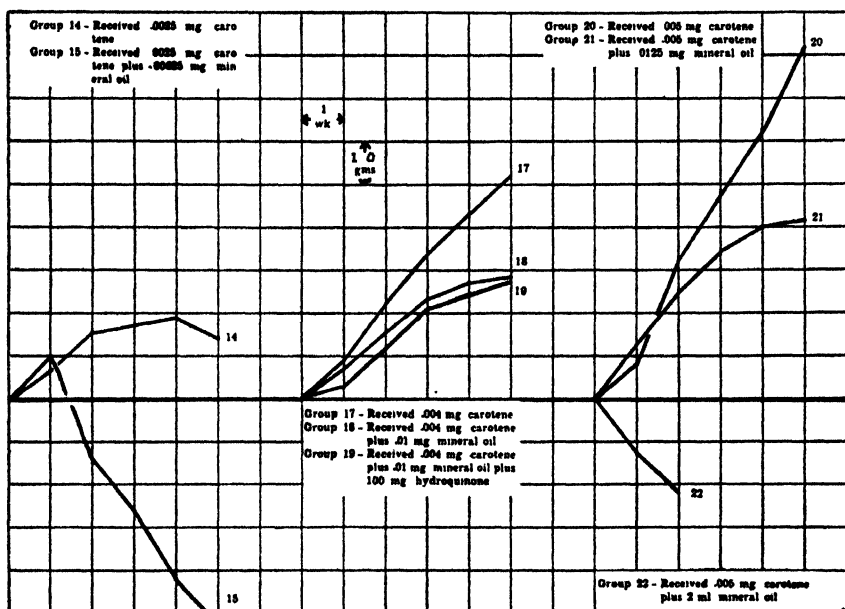


Chart 4

the presence of 5  $\gamma$  of carotene (group 22), all animals died within a period of 2 weeks with the usual symptoms of vitamin A deficiency.

When cod liver oil was fed daily (chart 5) at a level of 0.6 mg., which was barely sufficient for maintenance of body weight, there was some evidence that vitamin A utilization was lessened by the presence of 500 mg. of mineral oil. When the dosage of cod liver oil was increased to 1 mg. (daily), which was sufficient for growth, the presence of 500 mg. of mineral oil showed no visible effect on growth response



(groups 25 and 26). Similar results were obtained with groups 27 and 28 when the daily allotments of cod liver oil and mineral oil were increased to 2 mg. and 2 ml., respectively.

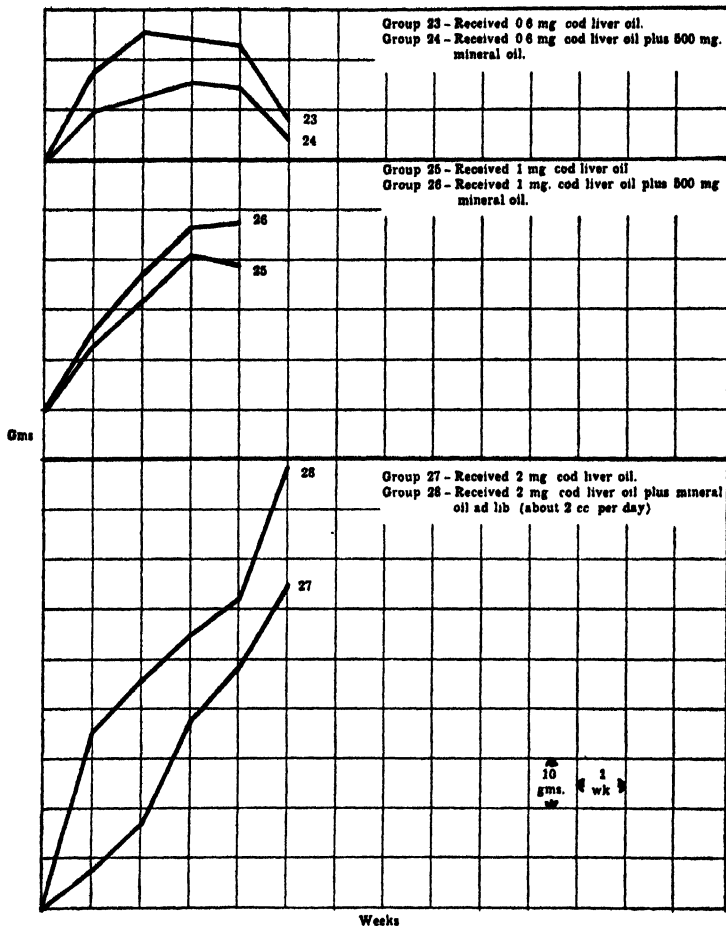


Chart 5

When a potent vitamin A concentrate was fed at a level approximating one Sherman unit (chart 6) in the presence and absence of varying amounts of mineral oil, no lowering of the vitamin A utilization could be noted.

It would appear, therefore, that carotene cannot be utilized when ingested in the presence of relatively small amounts of

mineral oil, while vitamin A (per se) is utilized quite efficiently when fed under similar conditions. Since hydroquinone had no tendency to improve utilization in the presence of mineral oil, it would appear that the lack of utilization is not due to oxidative destruction, which was postulated by Olcott and Mattill ('31).

All evidence seems to support the theory that mineral oil possesses a preferential solubility for carotene, preventing its absorption by removing it in solution from the digestive tract. In an endeavor to obtain additional evidence in support of this hypothesis, a second series of experiments was conducted.

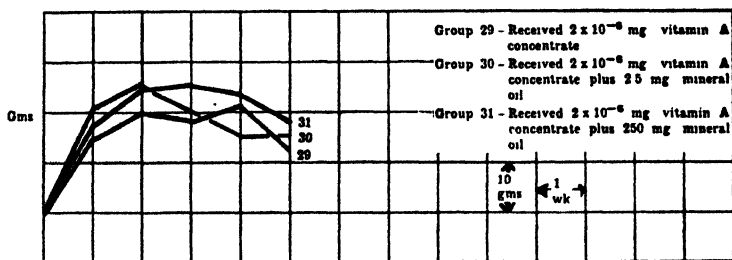


Chart 6

#### THE SECOND SERIES OF EXPERIMENTS

Prior to this work we had made repeated attempts to separate carotene and vitamin A from feces extracts obtained from rats which received these substances in mineral oil, with the view of re-feeding the carotene or vitamin A thus obtained, to prove, by biological response methods, that these substances are actually excreted in appreciable amounts. None of these attempts yielded satisfactory information owing to our inability to completely remove the mineral oil in order that it might not again vitiate results in the re-feeding period. Some encouraging data were obtained by adsorbing extracts in silica gel and similar adsorbants and by subsequent extraction of the adsorbant with various solvents (Zimmerman and Lachat, unpubl. data). None of these experiments was sufficiently quantitative for our purpose.

We also attempted to solve the problem by feeding varying amounts of carotene and vitamin A concentrate in the presence and absence of mineral oil, using 0.5 ml. of corn oil daily as in series 1. These feeding trials were of the prophylactic type in which the different groups of rats received carotene and vitamin A concentrate from the beginning of the experiment. Feces from these groups were collected daily and preserved under ether and the ether extracts were tested for carotene content with, a) the Lovibond tintometer, b) a colorimeter using a dichromate solution standardized against pure carotene, c) antimony trichloride color measurements, and, d) by spectroscopic measurements. These results were inconclusive, due to the pigmentation of the feces extracts with corn oil pigments and to other interfering substances. We also obtained evidence, by means of antimony trichloride tests and by rat assay methods, that our vitamin A concentrate was quite unstable in the new supply of corn oil.

As a result of these findings, we substituted ethyl laurate for corn oil and repeated the experiments, keeping the ratio of solvent to solute constant. The amounts of carotene, vitamin A concentrate and solvents are summarized in table 1.

Feces were collected daily from groups 32, 33, 34 and 35. These were preserved under petroleum ether during three periods of 10 days each. At the end of each 10-day period the feces were ground in petroleum ether and extracted with this solvent until no more color could be removed. These extracts were evaporated nearly to dryness and were made up to a volume of 10 ml. with redistilled chloroform.

Color values were obtained by comparing the color of the feces extracts with a standard dichromate solution in a colorimeter. No attempt was made to standardize this dichromate solution in terms of carotene, since we were interested primarily in showing whether or not a rough parallelism existed between increased carotene intake and increased carotene excretion.

The relative amounts of yellow pigment excreted during the increasing carotene intakes in the first 10-day period

are indicated graphically in chart 7. Data for the relative amounts of yellow pigment excreted during the second and third feces collection periods are omitted, since they were practically identical with those obtained during the first 10-day period.

TABLE 1

Group 32 Carotene in ethyl laurate	No. of animal	1	2	3	4	5	6
	Amount of carotene	.001 mg.	.002 mg.	.003 mg.	.004 mg.	.005 mg.	.006 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.
Group 33 Carotene in min- eral oil	No. of animal	1	2	3	4	5	6
	Amount of carotene	.001 mg.	.002 mg.	.003 mg.	.004 mg.	.005 mg.	.006 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.
Group 34 A concen- trate in ethyl laurate	No. of animal	1	2	3	4	5	6
	Amount of A concentrate	.01 mg.	.02 mg.	.03 mg.	.04 mg.	.05 mg.	.06 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.
Group 35 A concen- trate in mineral oil	No. of animal	1	2	3	4	5	6
	Amount of A concentrate	.01 mg.	.02 mg.	.03 mg.	.04 mg.	.05 mg.	.06 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.

In addition to the colorimetric evidence submitted in chart 7, spectrophotometric determinations of carotene were made on two feces extracts <sup>1</sup> using a Konig-Martens spectrophotometer. The samples used were extracts from groups 32 and 33, which received 6  $\gamma$  of carotene in ethyl laurate and mineral oil, respectively. The values obtained for  $-\log_{10}$  of the transmittancy of 2 cm. were 0.41 for the extract from group 32 and 1.49 for group 33, which, according to the graph of

<sup>1</sup> The writers are indebted to Dr. L. J. Briggs, director of the Bureau of Standards, Washington, D. C., for the spectrophotometric determinations of the carotene in these samples.

Schertz ('23), would correspond to 1 and 4 mg. of carotene per liter, respectively.

These values cannot be accepted as quantitative due to the fact that Schertz' measurements were made on carotene dis-

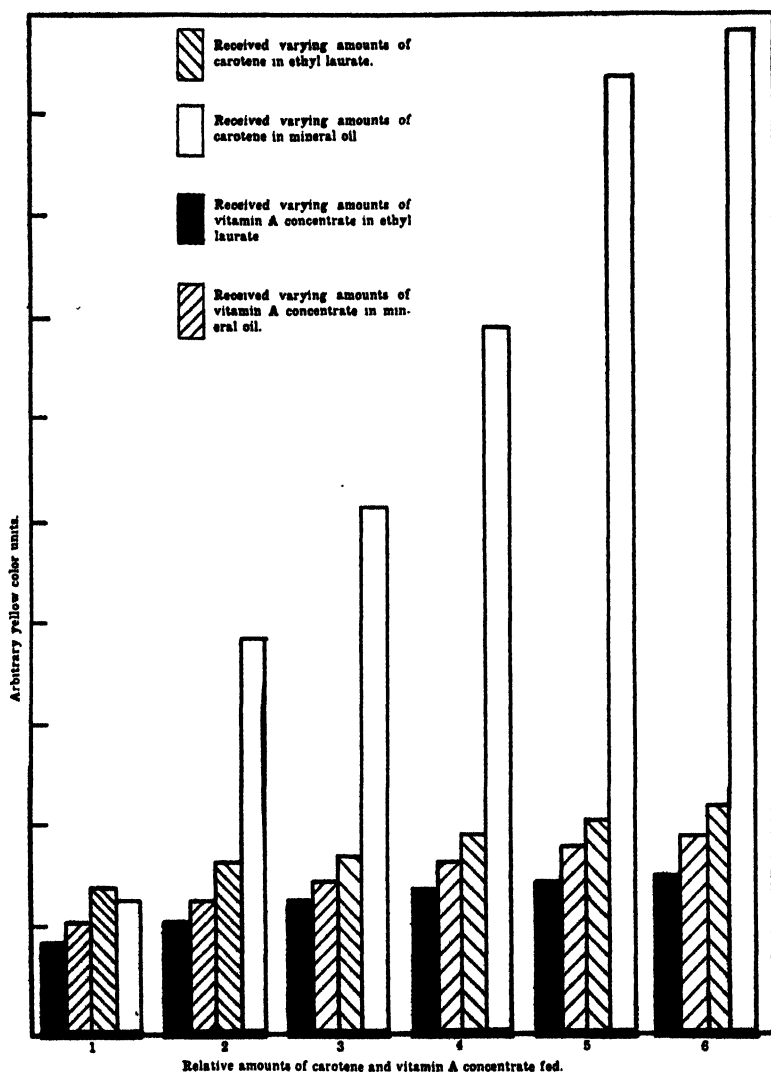


Chart 7 Showing relative intensity of feces extracts when rats received increasing amounts of carotene and vitamin A concentrate in ethyl laurate and mineral oil, respectively.

solved in ether, while our extracts were dissolved in chloroform. It is also certain that our extracts contained interfering substances. Since, however, the feces of both groups of animals were extracted under identical conditions, it would appear that the spectrophotometric data support the conclusion drawn from the color excretion data, viz., that the utilization of carotene is prevented by being excreted from the body in the unassimilated mineral oil.

### *Discussion of second series of experiments*

The question as to the mechanism whereby mineral oil prevents the utilization of carotene is answered in the second series of experiments. Having eliminated, to our own satisfaction, the possibility of oxidative destruction of carotene by a pro-oxidant in mineral oil, we felt that the detrimental effect might be explained by a lack of carotene utilization due to loss from the body by being voided in the unabsorbed mineral oil.

This hypothesis is supported by data obtained when the yellow color was determined in feces from rats which received increasing amounts of carotene in ethyl laurate in the presence and absence of mineral oil. The curve of pigment excretion (group 33) indicates that color excretion was almost in direct proportion to the amount of carotene fed, indicating that the utilization of carotene was almost completely prevented by the mineral oil. Spectrophotometric determinations of carotene in the feces extracts also supported the above conclusion. This was indicated also in the growth response of the rats in groups 32 and 33. We noted also that the growth response in the ethyl laurate groups was somewhat inferior to that obtained in the corn oil groups. This has been observed by other workers.

## CONCLUSIONS

1. Our previous results, showing that the vitamin A potency of butter fat is lowered, when fed at low levels in the presence of mineral oil, have been confirmed.

2. When butter fat was fed at higher levels in the presence of relatively small amounts of mineral oil, the deleterious effect of mineral oil was less marked, although the effect could still be noted.

3. The vitamin A potency of cod liver oil and of a cod liver oil concentrate was not adversely affected by the presence of mineral oil, which undoubtedly explains the less harmful effects of mineral oil on butter fat at the higher feeding levels, since a part of the potency of butter fat is due undoubtedly to vitamin A per se, while only a part of the potency can be ascribed to carotene.

4. The harmful effect of mineral oil can be explained on the basis of carotene excretion from the body in the unabsorbed mineral oil. This hypothesis is supported by the fact that yellow pigment excretion (when mineral oil is fed) is roughly proportional to the carotene ingested. This is not true when carotene is fed in the absence of mineral oil. Spectrophotometric determinations of carotene in the feces extracts also support this conclusion.

5. The hypothesis is advanced that the hydrocarbons of the unassimilated mineral oil possess a greater solvent effect on the hydrocarbon carotene than is possessed by the lipids of the intestinal juices, thereby preventing intestinal absorption of carotene. Conversely, it is suggested that the lipids and sterols of the digestive juices possess a preferential solvent effect on the sterol vitamin A, thereby promoting utilization by removing this vitamin from the unassimilated mineral oil.

## LITERATURE CITED

- BURROWS, M. T., AND W. K. FARR 1927 The action of mineral oil per os on the organism. *Proc. Soc. Exp. Biol. and Med.*, vol. 24, p. 719.
- DRUMMOND, J. C., H. J. CHANNON, AND K. H. COWARD 1925 Studies on the chemical nature of vitamin A. *Biochem. J.*, vol. 19, p. 1047.
- DUTCHER, R. A., J. O. ELY, AND H. E. HONEYWELL 1927 Vitamin studies. XV. Assimilation of vitamins A and D in the presence of mineral oil. *Proc. Soc. Exp. Biol. and Med.*, vol. 24, p. 953.
- DUTCHER, R. A., AND J. H. KRUGER 1926 Vitamin studies. XIV. The influence of ultra-violet light on the antirachitic properties of purified rations used in the study of vitamin A. *J. Biol. Chem.*, vol. 69, p. 277.
- HONEYWELL, H. E., R. A. DUTCHER, AND J. O. ELY 1931 Vitamin studies. XVIII. The biological assay of food materials for vitamin A as influenced by yeast from various sources. *J. Nutrition*, vol. 3, p. 491.
- JACKSON, R. W. 1931 The effect of mineral oil administration upon the nutritional economy of fat-soluble vitamins. I. Studies with the vitamin A of butter fat. *J. Nutrition*, vol. 4, p. 171.
- LACHAT, L. L. 1930 The adsorption of vitamin A and vitamin D on silica gel. Thesis, Pennsylvania State College.
- MCCOLLUM, E. V., AND N. SIMMONDS 1918 A study of the dietary essential, water-soluble B, in relation to its solubility and stability towards reagents. *J. Biol. Chem.*, vol. 33, p. 55.
- MONESS, E., AND G. CHRISTIANSON 1929 Assimilation of vitamin A when dissolved in liquid petrolatum. *J. Am. Pharm. Assoc.*, vol. 18, p. 997.
- MOORE, T. 1929 Vitamin A and carotene. I. The association of vitamin A activity with carotene in the carrot root. *Biochem. J.*, vol. 23, p. 803.
- 1929 Vitamin A and carotene. II. The vitamin A activity of red palm oil carotene. III. The absence of vitamin D from carotene. IV. The effect of various dietary modifications upon vitamin A activity of carotene. *Biochem. J.*, vol. 23, p. 1267.
- 1930 Vitamin A and carotene. V. The absence of the liver oil vitamin A from carotene. VI. The conversion of carotene to vitamin A in vivo. *Biochem. J.*, vol. 24, p. 692.
- OLCOTT, H. S., AND H. A. MATTILL 1931 The unsaponifiable lipids of lettuce. III. Antioxidant. *J. Biol. Chem.*, vol. 93, p. 65.
- ROWNTREE, J. I. 1931 The effect of the use of mineral oil upon the absorption of vitamin A. *J. Nutrition*, vol. 3, p. 345.
- SCHERTZ, F. M. 1923 The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. *J. Agr. Research*, vol. 26, p. 383.
- ZIMMERMAN, W. I. 1931 The absorption of vitamin A by the rat as influenced by mineral oil. Thesis, Pennsylvania State College.





# THE APPETITE STIMULATING AND GROWTH PROMOTING PROPERTY OF LIVER<sup>1</sup>

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## INTRODUCTION

The value of liver in the diet from the standpoint of the supplementary value of its proteins, of its content of mineral elements and water-soluble vitamins B and G, and its therapeutic principles in pernicious anemia has long been recognized. The extent, however, to which liver supplements enhance the nutritive value of a diet which is considered to be complete according to our present knowledge has not received much attention until comparatively recently.

## REVIEW OF LITERATURE

The first evidence of a definite acceleration of growth as the result of feeding liver in addition to a complete purified diet was presented by McCay, Dilly and Crowell ('29). By feeding brook trout raw liver in amounts as small as 5 per cent of the diet, a marked acceleration in growth resulted.

Guha ('31), after investigating factors necessary for the normal nutrition of the rat, concluded that the liver from sheep and swine, as well as other dietary articles, contained a growth factor which he was unable to identify with any of the recognized nutrients.

Daggs ('31) concluded from a study of the requirements for lactation of the female dog, that ". . . liver may contain some hormone or lactation-promoting vitamin . . . ."

<sup>1</sup>Paper No. 1252 journal series, Minnesota Agricultural Experiment Station.

Mapson ('32) has likewise demonstrated the presence of a factor in beef liver that stimulates lactation in the rat and which accelerates growth and improves fertility. He was unable to identify the factor responsible for these beneficial results as the protein, the ash, vitamin B<sub>1</sub>, or vitamin B<sub>2</sub> of the liver.

Bahrs ('33) observed an effect upon growth and fertility from the feeding of liver similar to that reported by Mapson. She also found, as did Guha ('31) and Mapson ('32), that the growth response of the males was much greater than that of the females. In none of these reports are data given relative to food consumption or to the effect of the liver upon appetite. It seems highly probable that at least a part of the beneficial results secured from the liver supplement could be explained on the basis of increased food intake as the result of appetite stimulation. This contention receives support from the belief of Palmer and Kennedy ('30) that the growth stimulating effects of fresh lettuce and liver and carrots are due to the effect on food consumption.

Graham and Griffith ('33) observed that when pork liver was fed to rats, there was an increase in growth which was associated with increased food consumption. The authors suggest that the vitamin G in liver may be the cause of its growth stimulus and raise the question as to whether the vitamin G requirements of the rat have been met in the experiments of other investigators who have shown a definite growth stimulus from liver feeding.

It would appear that their own work is open to criticism in that their basal diets were devoid of vitamin G. It would be impossible to demonstrate the presence of a special growth factor in liver by such technic unless the growth rates are compared with growth on similar diets in which the vitamin G is known to be adequate.

That liver may be valuable in the human dietary for reasons other than those commonly given is evidenced by the work of Bartlett ('28) on anorexia in children. His clinical results lead him to suggest that "the liver diet contains some specific

substance which increases an appetite that is depleted and which renews one that is lost."

This cannot, however, be considered as proof that the lack of appetite was originally caused by a deficiency in the diet of a factor needed for appetite stimulation. Such a condition might result from the lack of nutrients needed for growth which could be corrected by the addition of liver.

#### EXPERIMENTAL

In connection with some rat (Johnson, '33) and swine (Ferrin and McCarty, '30) feeding trials the unusual palatability of liver meal was demonstrated, as well as its ability to make a swine ration palatable that had, heretofore, resisted all attempts to make it so, regardless of the combinations of supplements used. The inclusion of two parts of this product in the swine ration, in place of the two parts of tankage used in the protein supplemental mixture of the controls, increased feed consumption by approximately 27 per cent. In the search for another material that had similar properties, a beef muscle product was obtained which had been processed according to the same procedure as the liver meal. An experiment with rats (Johnson, '33), comparing the two products when mixed into diets to the extent of approximately 5 per cent, showed that both products had similar appetizing qualities.

In order to obtain some information as to the effect of these supplements, as well as of fresh liver, upon appetite and growth, the following procedure was instituted, using the rat as the experimental animal.

The basal diet used consisted of the following ingredients: dextrin, 65.5 per cent; purified casein, 20 per cent; lard, 10 per cent; and Hawk and Oser's ('31) salt mixture, 4.5 per cent. In addition, 500 mg. of yeast tablets that had been immersed in cod liver oil previous to feeding were given to each rat daily.

The groups and supplements fed are shown in table 1.

Ten rats, five males and five females, were used in each of groups 1 to 4. The basal diet was fed ad libitum. Group 5a consisted of four males and five females which were paired with four males and five females in group 5b. They were fed according to Mitchell and Beadles' ('30) paired-feeding method. The control rat of each pair received an additional amount of the basal diet equivalent to the dry matter contained in the 0.5 gm. fresh liver given to its pair mate. Each rat was started on experiment at 28 days, kept in an individual cage, and fed its basal diet in a feed pan and its additional supplements in a small dish. Pork liver was selected for this study because it was available at all times at the Animal Husbandry Meat Laboratory and because it was desired to determine whether or not pork liver would have an effect upon growth similar to beef liver, especially in view of the fact that in the majority of investigations beef liver had been fed. In groups 2 and 3, 0.15 gm. of the liver meal and beef muscle meal were fed because it was desired to feed the same weight of dry matter as was supplied by the 0.5 gm. of fresh liver.

#### *Results with pork liver, liver meal and beef muscle meal*

The average growth and food consumption per week of the rats in each group are shown in table 2.

A consideration of the data in table 2 reveals that the fresh liver supplement increased both food consumption and growth as compared to group 1, which received only the basal diet. The liver meal likewise had a favorable effect on growth and food intake, but not to the same extent as did the fresh liver. During the experiment it was interesting to note that while the liver meal was consumed readily the beef muscle meal was apparently relished more than was the liver product, as evidenced by the time required for the complete consumption of the two products. Since the beef muscle product was liked even better than the liver meal, but since it did not result in an improvement of growth, it would seem that liver

contains some factor that stimulates growth either directly through a stimulation of the growth processes or indirectly through a stimulation of appetite. The former assumption seems to be more plausible, for it would seem that the avidity

TABLE 1  
*Supplement additions to basal diet*

GROUP	ADDITIONAL DAILY SUPPLEMENTS
1	none
2	0.5 gm. fresh pork liver
3	0.15 gm. liver meal
4	0.15 gm. beef muscle meal
5 a	none
5 b	0.5 gm. fresh pork liver

TABLE 2  
*Food<sup>1</sup> consumption and growth data of rats in a study of the effect of pork liver, liver meal and meat meal upon appetite and growth*

WEEK	GROUP 1		2		3		4		5		
	Basal		Basal + 0.5 gm. fresh pork liver		Basal + 0.15 gm. liver meal		Basal + 0.15 gm. beef muscle meal		a Basal	b Basal + 0.5 gm. fresh pork liver	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1st	24.6	44.1	27.8	44.9	25.8	44.8	25.4	44.5	19.6	39.9	19.3
2nd	17.6	48.4	24.5	51.1	21.9	47.1	19.2	47.7	19.1	50.5	21.2
3rd	20.5	53.1	28.3	59.2	24.0	57.6	21.8	54.5	22.4	52.9	21.2
4th	22.5	53.2	26.3	63.0	25.1	60.1	22.9	52.8	20.2	48.8	20.2
5th	13.7	57.2	19.6	65.9	17.3	61.1	15.4	55.0	14.4	52.6	15.5
Total	98.9	256.0	126.5	284.1	114.1	270.7	104.7	254.5	95.7	244.7	97.4
Average for 5 weeks	19.78	51.20	25.30	56.82	22.82	54.14	20.94	50.90	19.14	48.94	19.48

<sup>1</sup> The data for food consumption for groups 5a and 5b include the additional food given to 5a to correct for the dry matter in the liver supplement given to 5b.

with which a material is eaten would be an indication of its ability to stimulate the appetite. The results of group 5 throw some light upon this question. The gains between the pair mates are equal for 1 week and, for the remaining 4 weeks,

favor the controls for 2 weeks and the liver fed rats the other 2 weeks. This fact and the fact that the differences are of small magnitude forces one to the conclusion that the liver did not act as a growth stimulant when food consumption was controlled and that, therefore, in groups 2 and 3 the beneficial results from liver feeding must have been due to appetite stimulation even though the liver was not mixed as part of the regular diet. The statistical analysis of the data of the paired experiment, given in table 3, shows that there is a probability of 0.87858, i.e., odds of approximately 7 to 1, that the mean difference in total gains between pair mates was due to the liver supplement.

TABLE 3

*Statistical<sup>1</sup> results of paired-feeding experiments with rats fed purified diet and purified diet plus 0.5 gm. of fresh liver daily*

MEAN DIFFERENCE BETWEEN PAIR MATES IN TOTAL GAIN IN WEIGHT, GRAMS	STANDARD DEVIATION	Z VALUE	PROBABILITY
+ 1.66	3.684	0.452	0.87858

<sup>1</sup> 'Student' ('08).

After the above experiments had been completed, a report by Seegers and Smith ('32) of an attempt to determine in what manner liver exerts its favorable influence upon growth came to the writer's attention. By the use of the paired-feeding method they found that rats receiving the liver supplement grew at a faster rate than the controls.

An inspection of their data reveals that while equal amounts of the basal diet were given to both groups there was apparently no adjustment made for the amount of nutrients given in the liver supplement. During the 40-day experimental period this would amount to approximately 20 gm. of dry material, and when taken into consideration greatly minimizes the significance of the difference in growth between the controls and those receiving the liver.

For future studies of this appetizing factor it was considered desirable to determine the relative content of it in

livers from different species as well as in livers from young animals as compared to older ones. Pork, beef and calf livers were selected for this study. In order to obtain samples of liver representative of these two species, portions of ten pork livers and ten beef livers were secured from a local packing plant. Because of the expense, only one calf liver was obtained. Each kind of liver was first frozen and then ground and thoroughly mixed. The mixtures from which the daily supplements were taken were kept frozen.

The groups and the supplements they received are given in table 4.

TABLE 4  
*Supplement additions to basal diet*

GROUP	ADDITIONAL DAILY SUPPLEMENT
1	none
2	0.5 gm. fresh pork liver
3	0.5 gm. fresh beef liver
4	0.5 gm. fresh calf liver
5 a	none
5 b	0.5 gm. fresh beef liver

Ten rats, five males and five females, were used in each group. The basal diet was fed ad libitum to groups 1 to 4. Group 5a consisted of five male and five female rats which were paired with five males and five females in group 5b. These were fed according to Mitchell and Beadles' ('30) paired-feeding method. The same procedure was followed in this experiment as has been described in the first experiment.

#### *Results with pork liver, beef liver and calf liver*

The average gain in weight and food consumption per week of the rats in each group are shown in table 5.

The data for the first four groups emphasize the desirable effect of the liver supplements upon growth and food consumption. The three kinds of liver apparently vary but little in their content of the factor responsible for these results. That the factor had no effect whatever upon growth when food intake was controlled is shown by groups 5a and 5b.



It is interesting to note that the male rats showed a greater response to the liver supplement than did the females. This is shown in table 6, which gives the averages of the controls and of those receiving the fresh liver in both experiments. The difference in growth of the males on the two diets, however, is due entirely to greater food consumption, for when food intake was controlled, as in the paired experiments,

TABLE 5

*Food<sup>1</sup> consumption and growth data of rats in a study of the effect of pork liver, beef liver and veal liver upon appetite and growth*

WEEK	GROUP 1		2		3		4		5		
	Basal		Basal + 0.5 gm. fresh pork liver		Basal + 0.5 gm. fresh beef liver		Basal + 0.5 gm. fresh veal liver		<sup>a</sup> Basal	<sup>b</sup> Basal + 0.5 gm. fresh beef liver	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1st	25.0	54.7	35.1	62.4	34.0	61.0	32.7	58.0	19.9	46.8	20.9
2nd	24.6	64.4	32.7	73.8	38.3	77.0	34.8	73.2	23.8	56.1	24.4
3rd	24.7	69.4	32.8	85.0	32.3	87.3	33.1	86.6	28.0	67.4	28.0
4th	20.4	69.6	27.2	88.2	30.0	91.9	28.5	85.3	22.2	66.9	19.9
5th	16.7	74.3	20.7	88.4	23.3	94.7	22.2	89.2	17.0	66.0	17.1
Total	111.4	332.4	148.5	397.8	157.9	411.9	151.3	392.3	110.9	303.2	110.3
Average for 5 weeks	22.28	66.48	29.70	79.56	31.58	82.38	30.26	78.46	22.18	60.64	22.06

<sup>1</sup> The data for food consumption for groups 5a and 5b include the additional food given to 5a to correct for the dry matter in the liver supplement given to 5b.

TABLE 6

*The average weekly gain in weight and food consumption of male and female rats<sup>1</sup> in the study of the effect of liver upon appetite and growth*

DIET	SEX			
	Males		Females	
	Gain	Food	Gain	Food
	gm.	gm.	gm.	gm.
Basal + Liver	33.73	79.10	20.17	68.83
Basal	22.67	60.90	18.91	56.26

<sup>1</sup> Basal + Liver, 21 males and 19 females.

Basal, 11 males and 9 females.

growth of those receiving the liver was no greater than that of the controls.

#### SUMMARY AND CONCLUSIONS

The effect of several meat supplements upon growth and appetite was studied with rats. In the first experiment fresh pork liver, when fed as a 0.5 gm. daily supplement to a purified diet, increased growth and food consumption as compared to the controls receiving only the basal diet. When food consumption was controlled by use of the paired-feeding method, the rats receiving the liver supplement did not grow significantly faster than the controls. Liver meal, when fed in amounts equivalent to the fresh liver, increased the growth rates of the rats receiving it to a lesser degree than the fresh liver. Beef muscle meal, although more appetizing than the liver meal, when fed as a supplement to the basal diet did not increase food consumption. This result, in view of the similar appetizing properties of the two products when mixed as parts of the diet, is evidence that the palatability of a food itself is not a measure of its ability to stimulate the appetite for other foods with which it is not mixed.

In the second experiment the appetizing properties of fresh pork liver, beef liver and calf liver and the effect upon growth of fresh beef liver when food intake was controlled, were studied. The three kinds of liver were found to increase food consumption and growth to a similar extent. The increased growth, however, is entirely the result of appetite stimulation as shown by the equal growth of controls and those receiving the beef liver when food intake was equalized.

Male rats show a decidedly greater growth response to fresh liver supplements than do females. This is due in part to a greater consumption of food by the males and also to the fact that they utilized the greater food intake much more efficiently than the females.

These experiments confirm the conclusion of Palmer and Kennedy ('30) and suggest a different interpretation of the growth resulting than from a so-called 'growth' factor. The factor should be referred to solely as an appetite factor.

## LITERATURE CITED

- BAHES, ALICE M. 1933 The effect of adding liver to the diet upon the growth and reproduction of rats. *Am. J. Physiol.*, vol. 105, p. 262.
- BARTLETT, W. M. 1928 An analysis of anorexia. *Am. J. Dis. Child.*, vol. 35, p. 26.
- DAGGS, R. G. 1931 Studies on lactation. I. Production of milk in the dog as influenced by different kinds of food proteins. *J. Nutrition*, vol. 4, p. 443.
- FERRIN, E. F., AND M. A. MCCARTY 1930 Feeding rye to hogs. Minn. State Agr. Exp. Sta., Animal Husbandry Division, unpublished data.
- GRAHAM, C. E., AND W. H. GRIFFITH 1933 Studies on growth. I. Growth factors in liver. *J. Nutrition*, vol. 6, p. 179.
- GUHA, B. C. 1931 Observations on certain factors necessary for the normal nutrition of the rat. *Biochem. J.*, vol. 25, p. 960.
- HAWK, P. B., AND B. L. OSER 1931 A modification of the Osborne-Mendel salt mixture. *Science*, vol. 74, p. 369.
- JOHNSON, D. W. 1933 The nutritive deficiencies of the rye grain. Doctorate dissertation, U. of Minn.
- MCCAY, C. M., W. E. DILLY, AND M. F. CROWELL 1929 Growth rates of brook trout reared upon purified rations, upon dry skimmilk diets, and upon feed combinations of cereal grains. *J. Nutrition*, vol. 1, p. 233.
- MAPSON, LESLIE WM. 1932 Evidence of the existence of a dietary principle stimulating general growth and lactation. *Biochem. J.*, vol. 26, p. 970.
- MITCHELL, H. H., AND JESSIE R. BEADLES 1930 The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins. *J. Nutrition*, vol. 2, p. 225.
- PALMER, L. S., AND C. KENNEDY 1930 The fundamental food requirements for the growth of the rat. VI. The influence of the food consumption and the efficiency quotient of the animal. *J. Biol. Chem.*, vol. 90, p. 545.
- SEEGBERS, W. H., AND H. G. SMITH 1932 Influence of raw and whole dried liver on food consumption and utilization. *Proc. Soc. Exp. Biol. and Med.*, vol. 30, p. 365.
- STUDENT 1908 The probable error of a mean. *Biometrika*, vol. 6, p. 1.

# THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY INDIVIDUAL NUTRIENT DEFICIENCIES

## II. THE EFFECTS OF VITAMIN B DEFICIENCY <sup>1</sup>

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### ONE FIGURE

(Received for publication February 14, 1934)

### INTRODUCTION

It is evident, in the light of the current literature, that vitamin B must serve important functions in connection with the utilization of nutriment—functions which reveal themselves through the profound disturbance of metabolism when the supply of this vitamin is inadequate.

Especially in relation to the intermediary metabolism of carbohydrate, and therefore to energy metabolism, does it appear that vitamin B plays a prominent role. Also, the pathological changes resulting from vitamin B deficiency, even if not directly related to the metabolism of energy and protein, may indirectly affect the disposition of such nutrients in various ways.

Graham and Griffith ('33) studied the effect of vitamins B and G on the utilization of food for growth, and based conclusions on the average caloric requirement per gram of gain in weight, by young rats. Their results, such as are comparable on the basis of equal and fairly adequate caloric intake, indicate an increased consumption of food calories per gram

<sup>1</sup> Publication authorized by the director of the Pennsylvania Agricultural Experiment Station, February 13, 1934, as technical paper no. 635.

of total body gain, in case of rations depleted of both vitamins B and G. The evidence is less conclusive with regard to rations low in either vitamin B or G alone.

Evidence exists in the growth data recently presented by Sure, Kik, Walker and Smith ('33), of an effect of vitamin B deficiency on the utilization of food energy. On the same food intake rats on deficient and supplemented diets differed as much as 50 gm. in weight as a result of a growth period of about 15 weeks. Mitchell ('33) also presented data from which he concluded that rats which received a vitamin B supplement grew faster than did their pair-mates, whose food differed only in that it contained less of this factor. A question is raised as to the significance of these findings, however, by growth curves presented by Kon ('29), indicating that the average growth of groups of three rats each, fed a vitamin B deficient diet, is generally similar to the average growth of groups of rats fed an equal quantity of a complete diet; also by experiments of Hogan and Pilcher ('33), using systems of controlled and ad libitum feeding, which seem to show that the growth of rats deprived of the vitamin B complex does not differ significantly from others consuming equal quantities of a complete diet; and further by the results of the experiment to be discussed.

Conclusions as to effects of dietary deficiencies, based on growth data alone, are often compromised as a result of differences in food intake, and are also rendered uncertain because of lack of evidence as to the composition of the body increase. Also, in many experiments involving vitamin B deficiency, difficulty arises from the fact that it is impossible to distinguish effects due primarily to loss of appetite from others resulting from the nutritive defect itself.

The primary purpose of the experiment to be discussed was to observe the effect of vitamin B deficiency on the utilization of energy and protein, but secondary effects are also reported. The experimental subject was the albino rat; the paired method of food control was employed; and conclusions were based on metabolism, growth and body analysis experiments.

## EXPERIMENTAL PROCEDURE

The details of technic were as in the first of this series of papers (Swift, Kahlenberg, Voris and Forbes, '34), twelve pairs of rats being fed, for a period of 13 weeks, on a synthetic ration of the following composition: vitamin-free casein 18 per cent, Osborne and Mendel salt mixture 4 per cent, crisco 10 per cent, dextrin 64 per cent, cod liver oil 2 per cent, cellulose flour 2 per cent. This ration contained 93.5 per cent dry matter, 2.51 per cent nitrogen, and 4632 small calories per gram. Autoclaved brewer's yeast was fed separately, as a source of vitamin G and other heat-stable yeast factors. It analyzed 94.0 per cent dry matter, 8.21 per cent nitrogen and 4267 small calories per gram. Vitamin B was fed in the form of a special concentrate prepared and donated by Parke, Davis & Co. The potency of this concentrate, in terms of the Sherman unit was given, by Parke, Davis & Co., as one unit per milligram.

The basal ration was fed in equal quantities to each rat of a pair, the rat consuming the smaller quantity of food determining the intake of its pair mate. No refused food was discarded, the food intake of the last day of each weekly period being so restricted as to equalize the intake for the week, and so that no food remained for that day. Thus the weekly intake was kept the same for each rat of a pair.

The autoclaved yeast and vitamin B concentrate were weighed separately in quantities sufficient for a weekly allotment. The approximate daily allowance was then taken from these measures and added to each day's food. The intake of autoclaved yeast, which was kept the same for the two rats of each pair, varied from 0.2 gm. per day at the start to 0.5 gm. per day after about the fourth week. It represented about 10 to 15 per cent of the total food consumed, a quantity considered adequate for normal nutrition.

The rat of each pair which received the supplemented diet was given 3 to 4 Sherman units of the B-factor daily, while its pair mate received an occasional unit per day, as required to maintain slight but continuous growth. Aside from this

difference in vitamin B content the two diets were identical in composition.

Additional assurance of the fact that the basal diet was deficient in vitamin B is contained in the record of food refusals. Among all the pairs of rats the individuals on the deficient diet averaged 48.9 refusals for the entire experiment, as compared with an average of 7.3 refusals for the rats on the supplemented diet. The loss of appetite by the individual on the deficient diet became apparent by the end of the second week of feeding.

The general care and management of the rats, as well as the method followed in making the daily collections of urine and feces and in the preparation of these samples for analyses, has been previously described (Swift, Kahlenberg, Voris and Forbes, '34). At the end of 13 weeks, by which time most of the rats had at least doubled in weight, the rats were killed and analyzed in the manner reported in the foregoing reference.

The rectal temperature of each rat was taken on 3 consecutive days of each week during the last 3 to 5 weeks of the experiment. This was done at about 10 o'clock in the morning, before the day's food was given, and after water had been withheld for about 2 hours.

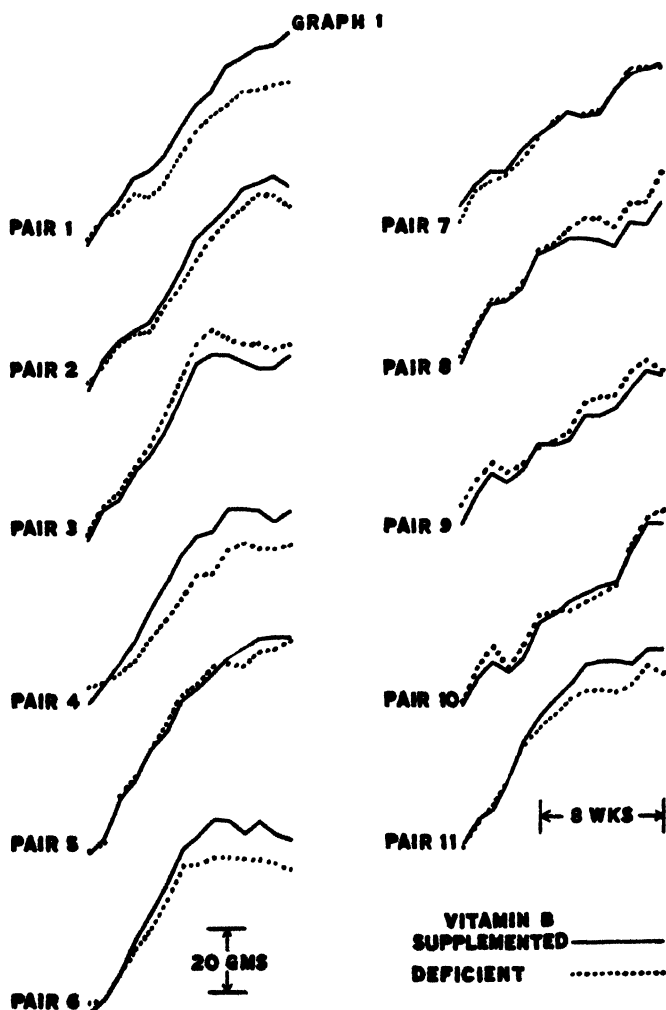
In reporting the data one pair of rats is omitted, because of an error in determining the sex.

#### RESULTS OF EXPERIMENT

##### *Growth*

Under the system of controlled food intake followed, it is possible to attribute differences in rate of growth of pair mates to the vitamin B deficiency alone. The growth curves appear in figure 1, and a comparison of the quantities of growth is contained in table 1. These data fail to reveal any consistent restriction of the rate of growth which may be attributed to a lack of vitamin B. The certainty of this conclusion, as it is related to the data in question, may be substantiated by statistical analysis. Among a possible 143

comparisons of weekly gains, the rat on the supplemented diet exceeded its pair mate (deficient diet) seventy-three times; the rat on the deficient diet exceeded its mate sixty-five times; and in five comparisons both rats gained the same.



An outcome such as this, among a series of events which may happen with equal probability in either of two ways, deviates but 4 from the ideal expectancy and is less than the calculated standard deviation.



In another view of the same data—the difference in weight gained or lost, per week, between the individuals of each pair of rats—it is found that the average of the 143 differences is  $+0.327 \pm 0.114$  gm., the plus sign indicating advantage in favor of the rats which received the supplemented diet. The odds are only 18 to 1 that this difference is significant and are insufficient to indicate that the supplemented diet was definitely superior for the production of gain in weight.

TABLE 1  
*Effect of vitamin B deficiency on growth and on food utilization*

PAIR NO.	SEX	FOOD INTAKE (DRY MATTER)	VITAMIN B DEFICIENT DIET				VITAMIN B SUPPLEMENTED DIET			
			Initial weight <sup>1</sup>	Final weight <sup>1</sup>	Gain	Dry matter of food for 1 gm. gain	Initial weight <sup>1</sup>	Final weight <sup>1</sup>	Gain	Dry matter of food for 1 gm. gain
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
1	F	383.9	48.46	97.65	49.19	7.8	46.83	118.88	67.05	5.7
2	F	347.4	43.62	98.10	54.48	6.4	42.08	105.74	63.66	5.5
3	M	367.5	45.30	103.44	58.14	6.3	43.61	99.90	56.29	6.5
4	F	343.8	46.83	89.23	42.40	8.1	42.13	98.54	56.41	6.1
5	M	380.3	45.34	108.24	62.90	6.0	44.50	110.34	65.84	5.8
6	M	337.3	46.97	88.47	41.50	8.1	44.07	96.76	52.69	6.4
7	F	343.3	43.69	90.86	47.17	7.3	48.32	89.39	41.07	8.4
8	M	348.9	41.43	99.12	57.69	6.0	39.43	90.57	51.15	6.8
9	M	327.0	53.49	96.19	42.70	7.7	47.49	92.43	44.94	7.3
10	F	339.6	42.06	99.50	57.44	5.9	41.14	93.81	52.67	6.4
11	M	363.1	45.70	98.58	52.88	6.9	46.91	106.20	59.29	6.1
Aver.		352.9	45.72	97.22	51.50	6.9	44.23	99.78	55.55	6.4

<sup>1</sup> Minus contents of alimentary tract.

#### BODY COMPOSITION

The composition of the bodies of the experimental rats, at the end of the feeding period, and of the gains in weight, appear in table 2. As a basis for estimating the composition of the rats at the beginning of the experiment, ten rats of the same age, which had received the same treatment as the subjects of the experiment, were killed and analyzed. The average results, expressed in relation to the empty fresh weight, are as follows: ether extract, 7.30 per cent; total nitrogen, 2.77 per cent; gross energy, 1.65 Cal. per gram.

The average per cent of alimentary 'fill' for these ten rats was 9.15 per cent, and this figure was used in estimating the initial empty weight of the experimental rats.

From the data of table 2 it is evident that the composition of body gains made was remarkably variable, and that growth data by themselves are not critically significant as measures

TABLE 2

*Effect of vitamin B deficiency on the ether extract, nitrogen, and energy contents of the bodies of rats*

PAIR NO.	COMPOSITION OF BODIES OF RATS <sup>1</sup>			COMPOSITION OF BODY GAINS			TOTAL GAINS		
	Ether extract	Nitrogen	Energy	Ether extract	Nitrogen	Energy	Ether extract	Nitrogen	Energy
Vitamin B deficient diet									
	<i>Per cent</i>	<i>Per cent</i>	<i>Cals. per gram</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Cals. per gram</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Cals.</i>
1	6.34	3.44	1.78	5.39	4.11	1.91	2.65	2.02	93.9
2	9.57	3.32	2.05	11.40	3.76	2.37	6.21	2.05	129.4
3	4.46	3.47	1.63	2.24	4.02	1.62	1.30	2.34	94.2
4	7.40	3.37	1.87	7.50	4.03	2.11	3.18	1.71	89.5
5	6.17	3.44	1.75	5.36	3.91	1.83	3.37	2.46	114.8
6	3.49	3.48	1.53	-0.82	4.29	1.40	-0.34	1.78	58.1
7	6.39	3.47	1.83	5.53	4.11	1.99	2.61	1.94	93.8
8	8.06	3.39	1.91	8.62	3.83	2.10	4.97	2.21	120.9
9	4.75	3.47	1.68	1.57	4.36	1.71	0.67	1.86	73.0
10	8.67	3.17	1.94	9.68	3.45	2.15	5.56	1.98	123.5
11	4.26	3.57	1.61	1.63	4.25	1.58	0.86	2.25	83.7
Aver.	6.32	3.42	1.78	5.28	4.01	1.89	2.82	2.05	97.7
Vitamin B supplemented diet									
1	7.26	3.45	1.85	7.23	3.92	2.00	4.85	2.63	133.9
2	10.46	3.24	2.11	12.55	3.55	2.41	7.99	2.26	153.3
3	5.91	3.44	1.76	4.83	3.96	1.85	2.72	2.23	104.3
4	10.39	3.33	2.16	12.69	3.74	2.53	7.16	2.11	143.0
5	6.61	3.43	1.83	6.14	3.89	1.95	4.04	2.56	128.2
6	3.88	3.47	1.57	1.01	4.06	1.51	0.53	2.14	79.4
7	11.37	3.26	2.27	16.14	3.82	3.00	6.63	1.57	123.3
8	5.00	3.48	1.64	3.23	4.03	1.64	1.65	2.06	83.8
9	6.90	3.32	1.81	6.48	3.89	1.97	2.91	1.75	88.7
10	11.40	3.22	2.20	14.62	3.57	2.63	7.70	1.88	138.5
11	6.26	3.53	1.80	5.45	4.13	1.92	3.23	2.45	114.0
Aver.	7.77	3.38	1.91	8.22	3.87	2.13	4.49	2.15	117.3

<sup>1</sup> Minus contents of alimentary tract.

of nutritive values of diets. Thus, while it has been shown that a deficiency of vitamin B in all probability did not affect the rate of growth of the rats in this experiment, the data giving the composition of the bodies, and of the tissues produced during growth, do reflect significant effects of the deficient diet.

As a result of the low vitamin B intake the bodies of the rats which had received the deficient diet contained less fat and energy than did their pair mates. The ether extract averaged 6.32 per cent, and the gross energy 1.78 Cals. per gram, in the bodies of the rats which had received the deficient diet, as compared to 7.77 per cent ether extract and 1.90 Cals. per gram, in the bodies of the rats on the supplemented diet. The difference is more strikingly reflected in the calculated fat and gross-energy contents of the gains produced. The average ether extract in the gains of the deficient rats was 5.28 per cent, or 2.82 gm., while in the rats which received the supplemented diet the corresponding values were 8.22 per cent or 4.49 gm. The average energy in the gains of the deficient rats was 1.89 Cals. per gram, or 97.7 Cals gained; and in the rats which received the supplemented diet, 2.13 Cals. per gram, or 117.3 Cals. gained. Thus, the deficient rats made 37 per cent less gain in ether extract, and 17 per cent less gain in body energy than did the rats which received the supplemented diet.

The percentage of nitrogen in the body gains averaged 4.01 for the rats on the deficient diet and 3.87 for the rats on the supplemented diet, but this difference is entirely accounted for by the larger percentage of fat in the bodies of the latter group.

#### UTILIZATION OF ENERGY

The digestibility of energy-producing nutriment (table 3) was the same for both diets.

The metabolizable energy, as reported, is not corrected for the non-metabolizable energy of the protein stored. For the purpose of this experiment, the interpretation of which is based largely on comparative data, the metabolizable energy

refers to the energy intake minus the total energy lost in urine and feces. Energy losses in the urine were consistently larger for the deficient rats, the odds being about 800 to 1

TABLE 3

*Effect of vitamin B deficiency on the digestibility, and the metabolizable energy of the food, and on the loss of heat from the body*

PAIR NO.	ENERGY INTAKE	ENERGY OF FECES	ENERGY OF URINE	DIGESTIBLE ENERGY		METABOLIZABLE ENERGY		HEAT LOSS	
				Total	Per cent of intake	Total	Per cent of intake	Total	Per cent of intake
Vitamin B deficient diet									
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>		<i>Cals.</i>		<i>Cals.</i>	
1	1886.6	131.7	79.7	1754.9	93.0	1675.2	88.8	1581.3	83.8
2	1705.9	117.5	66.0	1588.4	93.1	1522.4	89.2	1393.0	81.7
3	1805.5	122.5	83.7	1683.0	93.2	1599.6	88.6	1505.4	83.4
4	1686.6	107.6	83.8	1579.0	93.6	1495.2	88.7	1405.7	83.3
5	1867.7	132.1	67.4	1735.6	92.9	1668.2	89.3	1553.4	83.2
6	1654.6	102.4	60.6	1552.2	93.8	1491.6	90.1	1433.5	86.6
7	1685.9	102.1	77.5	1583.8	94.0	1506.3	89.3	1412.5	83.8
8	1713.8	104.6	58.4	1609.2	93.9	1550.8	90.5	1429.9	83.4
9	1604.9	96.4	55.9	1508.5	94.0	1452.6	90.5	1379.6	86.0
10	1667.4	137.6	65.3	1529.8	91.7	1464.5	87.8	1341.0	80.4
11	1783.0	105.6	63.1	1677.4	94.1	1614.3	90.5	1530.6	85.8
Aver.	1732.9	114.6	69.2	1618.3	93.4	1549.2	89.4	1451.4	83.8
Vitamin B supplemented diet									
1	1886.6	119.7	70.2	1766.9	93.7	1696.7	89.9	1562.8	82.8
2	1705.9	116.1	62.3	1589.8	93.2	1527.5	89.5	1374.2	80.6
3	1805.5	117.0	63.2	1688.5	93.5	1625.3	90.0	1521.0	84.2
4	1686.6	112.0	63.2	1574.6	93.4	1511.4	89.6	1368.4	81.1
5	1867.7	122.9	66.4	1744.8	93.4	1678.4	89.9	1550.2	83.0
6	1654.6	122.4	55.7	1532.2	92.6	1476.5	89.2	1397.1	84.4
7	1685.9	106.9	64.7	1579.0	93.7	1514.3	89.8	1391.0	82.5
8	1713.8	111.9	55.8	1601.9	93.5	1546.1	90.2	1462.3	85.3
9	1604.9	104.5	51.8	1500.4	93.5	1448.6	90.3	1359.9	84.7
10	1667.4	115.0	62.4	1552.4	93.1	1490.0	89.4	1351.5	81.1
11	1783.0	115.4	48.6	1667.6	93.5	1619.0	90.8	1505.0	84.4
Aver.	1732.9	114.9	60.4	1618.0	93.4	1557.6	89.9	1440.3	83.1

that this difference is significant. Since no significant difference was observed in the fecal energy loss, the metabolizable energy was slightly less for the deficient rats than for their pair mates on the supplemented diet.

The energy loss as body heat was computed as that portion of the energy intake not recovered in urine, feces and body gain. Although in eight comparisons among the eleven the deficient rats showed a greater heat loss than did their pair mates, the average difference for the eleven pairs was equivalent to only half of 1 per cent of the gross energy of the diet. The odds are only 13.5 to 1 that this is a significant difference.

Of the 19.6 Calories difference in average gain, in favor of the rats on the supplemented diet, 8.8 Calories is accounted for by difference in the energy content of the urine, 0.3 Calorie by difference in the feces, and the remainder by difference in the heat loss.

Data showing the utilization of food energy, and the distribution of the body gains between fat and protein, are presented in table 4. The average energy intake required to produce 1 Calorie of energy gain was 18.6 Cals. for the deficient rats and 15.4 Cals. for the control rats. In other

TABLE 4

*Effect of vitamin B deficiency on the utilization and distribution of energy in body gains*

PAIR NO.	VITAMIN B DEFICIENT DIET						VITAMIN B SUPPLEMENTED DIET					
	Energy of body gain						Energy of body gain					
	Total	Per cent of metabolizable	As fat		As protein		Total	Per cent of metabolizable	As fat		As protein	
			Cals.	Per cent of total	Cals.	Per cent of total			Cals.	Per cent of total	Cals.	Per cent of total
1	93.9	5.6	25.0	26.6	68.9	73.4	133.9	7.9	45.4	33.9	88.5	66.1
2	129.4	8.5	58.4	45.1	71.0	54.9	153.3	10.0	75.3	49.1	78.0	50.9
3	94.2	5.9	12.4	13.2	81.0	86.8	104.3	6.4	25.7	24.6	78.6	75.4
4	89.5	6.0	31.1	34.7	58.4	65.3	143.0	9.5	68.3	47.8	74.7	52.2
5	114.8	6.9	32.9	28.7	81.9	71.3	128.2	7.6	36.9	30.3	89.3	69.7
6	58.1	3.9	-2.5	-4.3	60.6	104.3	79.4	5.4	5.2	6.5	74.2	93.5
7	93.8	6.2	25.2	26.9	68.6	73.1	123.3	8.1	63.3	51.3	60.0	48.7
8	120.9	7.8	46.3	38.3	74.6	61.7	83.8	5.4	15.1	18.0	68.7	82.0
9	73.0	5.0	7.0	9.6	66.0	90.4	88.7	6.1	27.4	30.9	61.3	69.1
10	123.5	8.4	51.7	41.9	71.8	58.1	138.5	9.3	71.7	51.8	66.8	48.2
11	83.7	5.2	7.9	9.4	75.8	90.6	114.0	7.0	31.8	27.9	82.2	72.1
Aver.	97.7	6.3	26.9	24.6	70.8	75.4	117.3	7.5	42.6	33.8	74.7	66.2

words, on the same energy intake, rats which received a diet deficient in vitamin B required 21 per cent more energy to produce one unit of energy in body gain than did the rats which received the supplemented diet.

A more specific influence of vitamin B deficiency as affecting energy metabolism is the increased carbon-nitrogen ratio (table 6) in the urine. Similar effects have been observed by Bickel ('24), and by Kon ('29).

In harmony with the higher ratio of carbon to nitrogen in the urine of the rats on the deficient diet was their consistently lower temperature. The body temperature of the rats which received the supplemented diet varied between 99.7°F. and 100.9°F.—which appears to be normal. The rats on the deficient diet, however, varied in temperature between 97.8°F. and 99.9°F., and averaged 1.2°F. below the temperature of the other group.

It is impossible to ascribe definite significance to the difference between the computed heat losses of the two groups of rats, but the low body temperature of the rats on the vitamin B deficient diet, associated with a high energy value and high carbon to nitrogen ratio in the urine, suggest that this deficiency exercises a fundamental depression of the oxidative processes of the organism.

In any case the fact remains that the vitamin B deficiency lowered the energy value of the diet—a result which supports the idea of Forbes ('32) that the maximum energy values of individual foods are not realized unless they are fed in nutritively complete rations.

#### NITROGEN UTILIZATION

The data of table 5 indicate a slightly greater apparent digestibility of nitrogen by the deficient rats as compared with the controls—averaging 90.21 per cent for the deficient rats and 89.43 per cent for those on the supplemented diet. This deviation, averaging 0.78 per cent in the percentage digestibility, seems very slight, but its calculated probable error is  $\pm 0.19$ , and, according to Student ('08), the odds are 75 to 1

that such a difference is significant; also the digestibility of the deficient diet was apparently greater than that of the supplemented diet in nine cases among the eleven. This unexpected result may have been due to differences, 1) in digestive secretions and residues from the same, 2) in bacterial growth and residues, or 3) in completeness of absorption of digested nutriment as a result of differences in rate of passage of food residues along the alimentary tract.

TABLE 5

*Effect of vitamin B deficiency on the digestibility and utilization of food nitrogen*

PAIR NO.	NITROGEN INTAKE	VITAMIN B DEFICIENT DIET					VITAMIN B SUPPLEMENTED DIET			
		Nitrogen in feces	Nitrogen digested		Nitrogen intake recovered as body gain	Nitrogen in feces	Nitrogen digested		Nitrogen intake recovered as body gain	
			Gm.	Per cent of intake			Gm.	Per cent of intake		
1	12.56	1.31	11.25	89.6	16.08	1.17	11.39	90.7	20.94	
2	11.58	1.22	10.36	89.5	17.70	1.25	10.33	89.2	19.52	
3	12.12	1.19	10.93	90.2	19.31	1.25	10.87	89.7	18.40	
4	11.60	1.17	10.43	89.9	14.74	1.31	10.29	88.7	18.19	
5	12.58	1.21	11.37	90.4	19.55	1.36	11.22	89.2	20.35	
6	11.42	1.06	10.36	90.7	15.59	1.25	10.17	89.1	18.74	
7	11.39	1.05	10.34	90.8	17.03	1.14	10.25	90.0	13.78	
8	11.54	1.12	10.42	90.3	19.15	1.19	10.35	89.7	17.85	
9	10.95	1.09	9.86	90.0	16.99	1.25	9.70	88.6	15.98	
10	11.29	1.19	10.10	89.5	17.54	1.17	10.12	89.6	16.65	
11	12.04	1.00	11.04	91.7	18.69	1.31	10.73	89.1	20.35	
Aver.	11.73	1.15	10.59	90.2	17.49	1.24	10.49	89.4	18.25	

St. Julian and Heller ('31) found no effect of vitamin B deficiency on digestibility of protein.

The percentage of the intake nitrogen recovered as body gain is dependent on the total gain of nitrogen by the animal during the experimental period, since the nitrogen intake was the same for both rats of a pair. The data in table 5, showing the percentage of nitrogen intake present in the body gain, are based on the computed gains of nitrogen reported in table 2. The portion of intake nitrogen recovered as body

gain averaged 18.25 per cent for the control rats and 17.49 per cent for the deficient rats, this difference being not statistically significant.

TABLE 6

*Effect of vitamin B deficiency on the carbon-nitrogen ratio in urine*

PAIR NO.	VITAMIN B DEFICIENT DIET			VITAMIN B SUPPLEMENTED DIET		
	Carbon	Nitrogen	C: N	Carbon	Nitrogen	C: N
	<i>Gm.</i>	<i>Gm.</i>		<i>Gm.</i>	<i>Gm.</i>	
1	7.54	8.65	0.87	6.37	8.33	0.76
2	6.00	7.92	0.76	5.72	7.77	0.74
3	7.80	8.02	0.97	5.45	8.26	0.66
4	7.28	8.22	0.89	5.95	7.55	0.79
5	5.89	8.15	0.72	5.71	8.17	0.70
6	5.24	7.74	0.68	5.04	7.59	0.66
7	6.97	7.83	0.89	5.67	8.38	0.68
8	5.19	7.61	0.68	4.78	7.71	0.62
9	4.60	7.51	0.61	4.34	7.27	0.60
10	5.65	7.76	0.73	5.40	7.85	0.69
11	5.19	8.11	0.64	3.85	7.81	0.49
Aver.	6.12	7.96	0.77	5.30	7.88	0.67

#### SUMMARY AND CONCLUSIONS

The effects of vitamin B deficiency on the utilization of food energy and protein were studied in growth, metabolism and body analysis experiments, by the paired feeding method, with the albino rat.

Observations were made, 1) of the dry matter, ether extract, nitrogen and energy in the bodies of the experimental animals, 2) of the nitrogen, carbon and energy of the urine, 3) of the nitrogen and energy of the feces, and 4) of the body temperatures.

The effects of vitamin B deficiency were: 1) a specific depressing effect on appetite, but no certainly significant influence on gain in weight per unit of food; 2) a decided decrease in the quantities of fat and energy gained; 3) a lower body temperature; 4) a slight increase in digestibility of protein, but no effect on the digestibility of energy-producing nutriment; 5) a diminished efficiency of utilization of metabo-



lizable energy for body gain; 6) an increased energy outgo, as urine and heat, and an increased ratio of carbon to nitrogen, in the urine; and 7) an apparent depression of the oxidative processes of the organism.

## LITERATURE CITED

- BICKEL, A. 1924 Das Wesen der Avitaminose. Nach experimentellen Untersuchungen über die Abmagerungsform dieser Krankheit. *Biochem. Zeit.*, Bd. 146, S. 493.
- FORBES, E. B. 1932 Net energy of completely balanced nutriment as a measure of nutritive value of rations and of nutritive requirement of animals. *Am. Soc. An. Prod., Proc. Twenty-fifth Ann. Meeting*, p. 32.
- GRAHAM, C. E., AND W. H. GRIFFITH 1933 Studies on growth. II. The effect of vitamins B and G on the consumption and utilization of food. *J. Nutrition*, vol. 6, p. 195.
- HOGAN, A. G., AND R. W. PILCHER 1933 Effects of variations in the amounts of vitamin B and protein in the ration. *Mo. Res. Bul.* no. 195.
- KON, S. K. 1929 On the carbon:nitrogen ratio in the urine of rats deprived of one or both factors of the vitamin B complex. *J. Nutrition*, vol. 1, p. 467.
- MITCHELL, H. H. 1933 An application of the paired-feeding method to the quantitative estimation of the relative vitamin B contents of foods and artificial concentrates. *Am. J. Physiol.*, vol. 104, p. 594.
- ST. JULIAN, R. R., AND V. G. HELLER 1931 The effects of vitamin deficiency upon the coefficients of digestibility of protein, fat and carbohydrate. *J. Biol. Chem.*, vol. 90, p. 99.
- STUDENT 1908 The probable error of a mean. *Biometrika*, vol. 6, pp. 1-25.
- SURE, B., M. C. KIK, D. J. WALKER AND M. E. SMITH 1933 Dietary requirements for fertility and lactation. IV. The specific effect of vitamin B on lactation and growth. *Ark. Agr. Expt. Sta. Bul.* 284.
- SWIFT, R. W., O. J. KAHLBERG, LEROY VORIS AND E. B. FORBES 1934 The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. I. The effects of cystine deficiency. *J. Nutrition*, vol. 8, p. 197.

# THE FAT-SOLUBLE VITAMINS AND DENTAL CARIES IN CHILDREN <sup>1</sup>

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To test the effect on the progress of dental caries of fat-soluble vitamins, a group of nearly 500 seventh grade pupils was examined minutely, by clinical and x-ray exposure of every tooth. Approximately half of the number were then given for over a year extra vitamins A and D in tablet form (Squibb's Adex). The daily dose for each child was not less than 6000 U.S.P. vitamin A units and 1400 Steenbock units of D. Frequent visits by a social worker assured their consumption superimposed on the regular home diet. The other half of the children from the same social stratum served as controls. The experimental group finished with 147 children, the control with 171. The average age and sex distribution was the same in both. At the final examination, equally thorough with the first, the experimental group showed no improvement in caries incidence as judged by: 1) average increase in percentage of affected teeth; 2) average percentage increase in number of cavities; 3) percentage increase in 'average caries figure,' based on degree of involvement. Without otherwise improving the diet, the administration of concentrates of vitamin A and D to children of this age (14 at end) and within a period of 1 year seems to have no appreciable effect on the carious process in teeth already erupted or which erupt during the experimental period.

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## REVIEW OF LITERATURE

Considerable difference of opinion still exists as to the value, in the diet of man and the lower animals, of an adequate supply of the fat-soluble vitamins A and D. In recent years it has become more or less generally admitted that these food factors do play an important role in the development of normal tooth and bone structures. The argument has centered more particularly upon the question as to whether or not these vitamins are of importance in the prevention or retardation of the progress of dental caries. It seems desirable, therefore, to preface the report of the present investigation with a brief résumé of the findings of the numerous other workers in this field.

In the earlier work vitamins A and D were studied together as vitamin A. Since their differentiation in 1921, the effects of each have been studied specifically and much work has been devoted to the study of their influence upon the calcification of the bones and various tooth structures.

As the factor which induces calcification, vitamin D has received the greatest amount of attention in this regard. Some work has, however, been done in relation to the effects of a deficiency of the A factor upon the dental tissues. As a result of extensive experimental work on the two vitamins, Mellanby ('30) is of the opinion that it is vitamin D which is largely responsible for the normal calcification of the dental and osseous tissues, whereas vitamin A primarily controls the development of the soft peridontal tissues. Bloch ('31) believes with Mellanby that vitamin A has no direct influence on the calcification of the teeth. On the other hand, Marshall ('27) has reported that a diet deficient in vitamin A fed to pregnant animals and puppies produced not only abnormal growth of the jaws and alveolar processes, and resulting irregularity and delayed eruption of the teeth, but also structurally defective enamel and dentine. Marshall ('28) has also demonstrated the development, after maturity, of caries in rats said to be due to vitamin A deficiency. Wolbach and Howe ('33), in a study of the effects of vitamin A

deficiency on rodents, have reported defective enamel structure and gross deformities in the incisors resulting from absent or deficient dentine formation. They consider that these effects together with the formation of denticles, pulp bone and cementicles "may reasonably be regarded in the human as vitamin A deficiency possibilities." Somewhat similar findings have been reported by Shibata ('31). Hess and Abramson ('31) do not attribute dental caries to a lack of vitamin A and Knowlton ('30) has reported failure to produce caries in young rats fed diets deficient in this vitamin.

Animal experimentation bearing upon vitamin D in relation to tooth structure and dental caries has been quite extensive. Perhaps the most noteworthy is that of Mellanby. In dogs, rats and rabbits she has demonstrated defective and poorly calcified dentine with numerous interglobular spaces which still persisted when vitamin A was added to a basal diet deficient in A and D. Furthermore, poorly calcified teeth were produced in the offspring by feeding the mother on a diet deficient in vitamin D. These findings led Mellanby to the conclusion ('29) that the anti-rachitic vitamin ranks first in importance in calcifying processes; and in view of the fact that the foods containing it are relatively few and expensive, a deficiency of this factor is more probable in the average diet than a deficiency of calcium and phosphorus great enough to interfere materially with the normal development of the dental tissues. In spite of the marked pathological changes in the dental tissues, attempts to produce caries in dogs failed. Some dentine softening, but no enamel or cement caries was found in rabbits. Only occasionally gross macroscopic lesions were found in the teeth of rats on a low vitamin D diet, but even in these, histological examination did not always disclose bacteria in the dentinal tubules.

Feeding the McCollum rachitogenic diet 3143 to rats for 7 to 42 days from the age of weaning, Becks and Ryder ('31) reported pathological changes in the dentine similar to those spoken of by Mellanby. These changes increased in extent and severity as the feeding period was prolonged. These

authors believe that the defective calcification of the dentine in rickets is primarily due to a progressive degeneration of the odontoblasts. On the same rickets-producing diet, Kronfeld and Barker ('32) found similar pathologic disturbances in the calcification of the teeth and bones and further reported that these changes may be prevented and influenced by the administration of a vitamin D preparation such as viosterol.

Corresponding disturbances in the calcifying mechanism on various rachitogenic diets have been reported by Shibata and other workers.

With the administration of vitamins A and D in the form of cod liver oil, Orban ('27) has reported a decided increase in growth of the incisors of rats. Karshan ('29) has been able to produce only slight variations in the phosphorus, calcium and total ash content in the incisor teeth of rats on rachitic diets causing marked changes in the composition of the bones.

Somewhat conflicting results have been obtained by various investigators who have attempted to produce experimental caries in animals maintained for periods upon diets deficient in vitamin D. Blackberg and Berke ('32 a) reported no enamel or dentine changes in the teeth of rats after 25 days on a strictly rachitogenic diet, but marked inflammation and degenerative changes in the pulp, occurring consistently. In more advanced stages of rickets dentine and enamel changes were observed, characterized by an increased amount of predentine and faulty calcification. In later work ('32 b) feeding 5-week old rats a modified Steenbock and Black diet no. 2965, the same investigators reported 33.3 per cent incidence of caries in the rachitic group, 100 per cent incidence in a group receiving excess viosterol and complete freedom from caries in a control group receiving the basal ration with the addition of a therapeutic amount of vitamin D.

Agnew ('33) has reported the development of caries in seventy out of seventy-one rats fed a diet low in vitamin D and phosphorus for periods of from 2 to 7 months. It is admitted that this diet was low in other minerals and vitamins

and the proteins were not of good quality. Moreover, it has been demonstrated elsewhere that there is a possibility of the initiation of the caries process by fracture through the agency of the hard corn particles which comprise a large proportion of the diet. Agnew believes that in the rat the role of vitamin D is of less importance than that of phosphorus. The results of his experiments would seem to justify this conclusion, but the work should be repeated in view of the inconsiderable number of animals in some of the experimental groups.

The work of Lilly ('32), of Knowlton ('30), and of Hoppert, Webber and Canniff ('32) would appear to indicate that a lack of vitamin D in the diet is not sufficient to produce caries, and that the administration of liberal amounts of this vitamin will not appreciably retard the decay of the teeth.

In view of the differences in metabolism, in the morphology of the teeth, and in the type of lesion produced, it is difficult to evaluate the results of this animal experimentation and to know just how far we are justified in applying these results to man. Much valuable information has been obtained from laboratory animals. We know that specific alterations or deficiencies in the diet will cause marked structural changes in the dental tissues. It has yet to be demonstrated that identical changes are brought about in the teeth of man during the development period or afterward. Furthermore, it has never been conclusively proved that the condition of hypoplasia, either microscopic or macroscopic, will cause a predisposition to dental decay. The difficulties of satisfactorily controlling such experiments on the human subject have necessarily limited the amount of direct experimentation in this field. Nevertheless, much valuable work has been accomplished.

It must be admitted that there is much evidence to support the hypothesis advanced by Mellanby and others that the control of dental caries is definitely correlated with vitamin D and calcium and phosphorus metabolism. Yet it is far from adequately established. The earlier work of Mellanby, Pattison and Proud ('24, '26, '28) suggested that the addition of

fat-soluble vitamins, principally D, retarded the development of carious cavities and brought about the arrest of active caries. This work was criticized on the score that the experimental period (7 months) was too short and that the children were all tuberculous and therefore already subject to abnormal calcium metabolism. Further work by the same investigators ('31) only served to confirm the earlier results. In the Sheffield investigation it was found that the percentage increase in degree of caries in the groups receiving supplements of olive oil, cod liver oil and radiostol, respectively, was 62.13, 10.28 and 6.57, while the corresponding figures in the Birmingham investigation were 45.85, 9.81 and 10.12. The conclusion was therefore reached that the incidence and rate of progress of caries in teeth already erupted can be lessened by the addition of fat-soluble vitamin D to the diet, inasmuch as the antirachitic vitamin is common to both the apparently effective substances.

The investigations of Weston Price ('32) point to the improved dental condition being due to general dietary improvement rather than to the addition of any specific factor. Price has reported the "control of dental caries to 90 per cent efficiency in even the worst cases" following the addition of fat-soluble activators coming from butter and cod liver oil with reinforcements of the minerals in milk and the mineral content of selected cereals. From 6 months' observation he reports ten times as many cavities in those not receiving activator concentrates.

The findings of McBeath ('33) would suggest that the dietary control of dental caries was limited in duration after the withdrawal of protective diets. He found quartz-mercury lamp radiation more effective than either viosterol or cod liver oil. It is difficult to reconcile these findings with the observation of many investigators in relation to the geographical distribution of dental caries. The disease is rampant in many tropical countries where the production of vitamin D through skin irradiation is at a maximum. Jones, Larsen and Pritchard ('30) report general disintegration of the teeth

of infants and small children in Hawaii despite a plentiful supply of vitamins, sunshine, cod liver oil, eggs, fruit juices and milk. Rickets is practically unknown in South Africa, yet 93 per cent of the children are said to have caries (Friel, Irel and Shaw, '31). From an experimental study of the teeth of children who had developed rickets in infancy, as compared with the teeth of normal children, both groups being closely observed during infancy and again at 6 or 7 years of age, Hess and Abramson ('31) have concluded that vitamin D is not the primary factor in the development of dental caries. Toverud and Toverud ('31) agree with Mellanby and others in reporting the occurrence of thin poorly calcified enamel, and interglobular spaces in the dentine following calcium, phosphorus and vitamin D deficiency during foetal life and during the period of lactation. Furthermore, in an unstated number of children examined, these investigators report high caries incidence with or without hypoplasia of the enamel where the data indicate an insufficient intake of mineral salts and vitamins (particularly D) during the developmental period both before and after birth. Toverud considers the metabolic disorder giving rise to hypoplasia (formed postnatally) is more likely to be due to deficient intake of vitamin D than to deficient mineral intake.

The conclusions reached by McKay and Rose ('31) are in conflict with those to be drawn from much of the work cited in relation to hypoplasia and dental caries. These conditions were contrasted in a group of children having the undoubted stigmata and history of past rickets, with a control non-rachitic group. It was found that gross hypoplasia of the permanent dentition was present in 22 per cent of the rachitic group and in only 2.5 per cent of the control group. This would seem to indicate a definite association between hypoplasia and vitamin D deficiency; yet the percentage of carious teeth was almost identical in both groups in both the deciduous and permanent dentitions. In the opinion of the authors, this evidence "strongly suggests, if it does not prove" that a deficiency of vitamin D in early childhood with



consequent hypoplasia, either microscopic or macroscopic, is not an important controlling factor in the incidence of dental caries. Unfortunately, the groups were small and it is impossible to rule out entirely the possible influence of subsequent antirachitic treatment upon the susceptibility of the teeth to caries. Moreover, it is impossible to estimate the proportion of cases of gross hypoplasia in each group which may have been due to metabolic disturbances arising from the various exanthemata of childhood.

Wieland ('31) has made the statement that more than 80 per cent of all infants under 1 year of age show signs of florid rickets. If this could be substantiated it would indeed be of the utmost importance to discover the true relation between rickets and hypoplasia and between hypoplasia and dental caries. Reporting on 100 children from 5 to 17 years of age suffering from rickets, varying from mild to severe, Wilson and Surie ('30) state that the degree of severity of rickets and caries appeared to run parallel. Fourteen children with mild rickets showed evidence of caries in 21 per cent of cases and of hypoplasia in 85 per cent, while all of the nine children with severe rickets showed both caries and hypoplasia.

The ratio of calcium, phosphorus and vitamin D in the diet would seem to be an important factor. It is known that the effects of Ca and P deficiency are less marked when there is much vitamin D in the diet. Similarly, less vitamin D is required to prevent rickets when there is an ample supply of calcium and phosphorus. Furthermore, an excess of either calcium or phosphorus may produce rickets.

Boyd, Drain and Nelson ('29, '31), Brodsky and Schoenthal ('33) and Bunting and his associates ('30) have all reported successful control of the caries process by dietary measures. It is important to note, however, that the results of these investigators were obtained not by the omission or inclusion of any one specific factor, but rather by the adoption of a dietary which was improved with respect to its mineral and vitamin content without the addition of mineral supplements

and sometimes without superimposing therapeutic food accessories.

Even those investigators who have stressed the value of the fat-soluble vitamins have in some instances effected improvements in the diet in relation to vitamins other than A and D and at the same time increased the mineral content. In the light of our present knowledge, it would seem reasonable to conclude that a regular diet, adequate in all essential factors is the best to follow. It is obvious, from a review of the literature, that it has not yet been definitely proved that the lack of any one factor is responsible for the high incidence of dental caries at the present day. Furthermore, the functions of digestion, absorption and intermediary metabolism, are not yet fully understood.

#### METHOD OF INVESTIGATION

The decisive test of the efficacy of the fat-soluble vitamins in arresting or retarding the progress of dental caries must be made on man himself. The work of Mrs. Mellanby suggests that not only teeth in the process of calcification, but those already formed may be rendered less susceptible to dental caries by the administration of an ample supply of these vitamins, mainly vitamin D.

It was therefore decided to conduct an investigation in an attempt to discover whether active caries could be arrested or significantly retarded by superimposing a generous supply of the fat-soluble vitamins upon the ordinary home diets of a group of school children. In view of the fact that the caries process is conceded to be most active around the age of puberty, it was felt that the application of these principles could be best studied with a group at the age of 13 years. Accordingly, permission was obtained to carry out the work in the seventh grade of one of the public schools of Rochester. Our original examination included the whole of this grade, numbering some 500 children of both sexes and of the average age of 13 years. This number was then reduced to some 430, in order to eliminate those much above or below the

average age and those from whom complete data were unobtainable for various reasons. The nature of the investigation was outlined and the cooperation of teachers, parents and children obtained. Of the 430 children, 220 children and their parents expressed a willingness to enter the experimental group and to cooperate for the necessary length of time. The remaining 210 constituted the control group. For the selection of the experimental group the only factor taken into consideration was the enthusiasm of the parents and children in their desire to cooperate. Subsequent investigation revealed that the two groups were as nearly as could be judged of the same economic status, with approximately equal proportions of the various nationalities. Furthermore, as will be seen from the data, the original dental condition of the two groups was practically identical. No attempt was made to interfere with, or influence in any way, the regular home diet of the children.

Each child in the experimental group received a daily ration of a fat-soluble vitamin concentrate in tablet form. The tablets were physiologically standardized, the vitamins being in a form readily assimilable. Each tablet contained not less than 1000 U.S.P. units of vitamin A and 245 Steenbock units of vitamin D. The daily ration for each child was six of the tablets which would be equivalent to three teaspoonfuls of high-grade cod liver oil, reenforced with viosterol. These were delivered at the homes periodically by an assistant (Mrs. Grace Simmons, A.B.) who was employed throughout the whole course of the investigation in order to maintain constant contact with the children and parents in their homes.

It was realized that this was the crucial factor in the investigation and our thanks are due to Mrs. Simmons for her enthusiasm and untiring efforts in this direction.

As is always the case in an investigation of this nature, it was inevitable that there should be considerable defection from the group for various reasons. Absence at one or other of the examinations or transfer from the school was accountable in part. Furthermore, if any signs of flagging interest

or irregularity in taking the concentrates were observed at any time during the course of the experiment, these children were immediately eliminated from the group. This was done in every case before the final examination and regardless of the dental condition. Although this action depleted the number of experimentals by approximately one-third, it assured us of a thoroughly reliable group. It was found that in the final assessment 147 children remained in the experimental group and 171 in the control group.

Three detailed dental inspections were made; the first in October, 1932, the second in May, 1933, and the third in December, 1933. Individual charts were used, the actual location and extent of each cavity or filling being recorded and additions made on attached charts at subsequent examinations. Cavities and fillings were differentiated by the use of different colored markings. Each child was submitted to a thorough prophylactic cleaning before examination by mouth mirror and explorer. A complete radiographic survey of every mouth was made in the first and final examinations. The discovery by this means of numerous incipient interproximal cavities at or just below the contact point, and consequently quite beyond the reach of the finest explorer proved the x-ray check-up to be not only advisable, but indispensable in investigations of this type. At the same time plaster of Paris models were made of the teeth in both upper and lower jaws for use in another series of observations.

The number of deciduous teeth remaining in the mouth at this age was very small and those still present had passed their allotted term. Although a record was kept of the condition of these teeth, it was therefore deemed advisable to disregard them entirely in our caries figure computations. All extracted teeth were regarded as having been carious except those which were known to have been lost through trauma or extracted for aesthetic or orthodontic purposes. In order to arrive at the total caries figure for each mouth and the average caries figure for the two groups, it was necessary to classify each cavity according to the extent of

the caries process. In the case of multiple caries in one tooth the summation of those numbers assigned to the individual cavities would give the caries figure for the tooth. Adding the caries figures for each tooth would give the total caries figure for the mouth. This divided by the number of erupted teeth would thus yield the average caries figure for the mouth.

It was therefore decided to adopt a modification of the method employed by Mellanby and assign numerical values as follows:

1. Initial caries including softened or discolored pits and fissures giving lodgment to a fine explorer; approximal caries subsequently verified by radiograph, but not freely accessible and small carious points in any part of the tooth.

2. Freely accessible approximal cavities and small open cavities involving less than one-fourth of the tooth.

3. More extensive caries involving more than one-fourth and less than two-thirds of the crown.

4. Caries involving from two-thirds to complete destruction of the crown.

Further data collected during the course of the investigation have been reported elsewhere.

#### PRESENTATION OF DATA AND DISCUSSION

It must be admitted at the outset that in order to have a satisfactory criterion of the arrest or retardation of the caries process in any given mouth or group, the rate of progress prior to the commencement of the experimental period should, theoretically, be specifically noted. However, this observation would be impracticable, or at least unreliable, for the reason that the relative increase in caries incidence is known to vary considerably as the age of the patient increases. Furthermore, it is recognized that the susceptibility to caries of the individual teeth varies. In the course of 12 months, which would be the minimum time required to make such an observation, the combination of teeth in any individual mouth may be greatly changed. It would therefore seem that the preliminary observations of this nature would be worthless.

The composition of the two groups retained for the final inspection is set out in table 1. It will be seen that the number of females in both groups was the same while there were more males in the control than in the experimental group. In view of the close agreement between the initial and final average caries figures for the male and female members of both groups, it is felt that this discrepancy cannot constitute an appreciable source of error. The average age of the controls and experimentals was very similar and extremely uniform. The children used in Mrs. Mellanby's investigations were between 5 and 14 years of age, the average being under 10 years.

TABLE 1

GROUP	NUMBER MALES	NUMBER FEMALES	TOTAL NUMBER	AVERAGE AGE AT END OF EXPERIMENT
I. Control	92	79	171	13 years 11 months
II. Experimental	68	79	147	13 years 10 months
Total both groups	160	158	318	

TABLE 2

GROUP	EXAMINED	TOTAL ERUPTED TEETH	TEETH ERUPTED DURING EXPERI- MENTAL PERIOD	NUMBER DECAYED	PER CENT DECAYED
I. Control	first	4501			
	final	4681	180	106	58.89
II. Experimental	first	3763			
	final	3968	205	122	59.50

Consequently, her observations dealt largely with deciduous teeth; and the daily ration of cod liver oil was varied arbitrarily according to the age of the child.

Table 2 shows the total number of teeth erupted at the first and final inspections, respectively, in the control and experimental groups. In the fourth column will be seen the number of fresh teeth erupted during the experimental period. The next two columns indicate, respectively, the number and percentage of the newly erupted teeth which had already decayed. The percentages in the two groups closely parallel each other. It is noteworthy that such a high proportion (almost 60 per cent) of these teeth so recently erupted were already defective.

From a study of table 3 it is apparent that there is no significant difference between the experimental and control groups in the incidence of dental caries computed on the basis of the number and percentage of individual teeth affected at the first and final examinations. Comparisons made on this basis are apt to be erroneous or misleading. This is

TABLE 3

GROUP	NUMBER CASES	EXAMINED	TOTAL TEETH ERUPTED	TEETH CARIOUS OR FILLED	PERCENTAGE CARIOUS OR FILLED	INCREASE IN PERCENTAGE OF AFFECTED TEETH
I. Control	171	first	4501	2507	55.47	5.77
		final	4681	2866	61.24	
II. Experimental	147	first	3763	2084	55.38	5.28
		final	3968	2407	60.66	

TABLE 4

GROUP	ADDITIONAL CAVITIES SINCE FIRST EXAMINATION		TOTAL	NUMBER CAVITIES FIRST EXAMINATION	PER CENT INCREASE	NEW CAVITIES PER MOUTH	TOTAL
I Control	upper	690	1158	3197	36.22	upper 4.04	6.77
	lower	468				lower 2.73	
II Experimental	upper	659	1106	2526	43.78	upper 4.48	7.52
	lower	447				lower 3.04	

TABLE 5

GROUP	EXAMINED	AVERAGE CARIES FIGURE	INCREASE IN AVERAGE CARIES FIGURE	PERCENTAGE INCREASE IN AVERAGE CARIES FIGURE
I. Control	first	1.024	0.335	32.71
	final	1.359		
II. Experimental	first	0.991	0.332	33.50
	final	1.323		

well illustrated by comparing this table with tables 4 and 5. There is no doubt that the number of cavities present is a better criterion for the true dental condition in relation to the caries process, than is the number or percentage of defective teeth.

These data are set fourth in table 4, together with the percentage increase over the original number of cavities and the average number of fresh cavities per mouth. A still more

reliable method for arriving at a true caries index for any mouth or group is the method, outlined earlier, for computing the average caries figure and taking into account the extent of the caries process as well as the number of carious points. The results of these calculations have been summarized briefly in table 5. It will be seen that the original average caries figure for the group receiving the fat-soluble vitamin concentrates was quite comparable to that of the control group, the difference being approximately 3 per cent. The average increase in caries figure for the two groups was almost identical; and the percentage increase in the average caries figure showed a very small variation, well within the limits of experimental error. Only fifteen children showed no increase in the caries figure between the first and final inspections. Of these, seven were in the control group and eight in the experimental group. None of these showed absolute freedom from new cavities, the apparent arrest being due to the eruption of new teeth during the experimental period, with at the same time only a slight increase in caries incidence.

It was recognized that in experimental work of this nature the personal equation should be eliminated in so far as it is possible. It was felt that a knowledge of the groupings of the children might unconsciously influence the findings of the operators to some extent. Therefore, at the final inspection care was taken that the operators were quite unaware as to whether the child being examined was in the control or the experimental group. Furthermore, the first examination was conducted before the partition was made and this was eventually effected regardless of the dental condition.

The radiographic survey proved an invaluable aid. Our experience in this regard leads us to the conclusion that, although it adds materially to the work involved in such an investigation, the Roentgen-ray check-up is quite indispensable if reliable results are to be obtained. This fact is obvious from a study of table 6. Of the total of 2264 additional cavities which developed subsequent to the first examination, no fewer than 620 would have been overlooked even with the most



careful oral examination. All of them were located in positions at the contact point quite inaccessible to the finest explorer. The table indicates that 28.67 per cent of fresh cavities in the control group and 26.04 per cent in the experimental group could be revealed only by the x-ray. This is an increase of 37 per cent over the original number of 1644 new cavities found with mouth mirror and explorer. In many cases the radiograph disclosed no additional cavities, whereas in others the apparent increase in the caries figure was increased as much as 200 per cent.

An interesting point brought out by this investigation was the continued tendency for those children showing an originally low caries incidence to maintain a lower absolute increase

TABLE 6

GROUP	TOTAL ADDITIONAL CAVITIES SINCE FIRST EXAMINATION	TOTAL	NUMBER REVEALED ONLY BY X-RAY SURVEY	TOTAL	PER CENT REVEALED ONLY BY X-RAY
I Control	upper 690 lower 468	1158	upper 173 lower 159	332	28.67
II Experimental	upper 659 lower 447	1106	upper 139 lower 149	288	26.04
Total (2 groups)	2264		620		27.35

in the average caries figure than those with a high caries figure at the beginning of the experimental period.

In the whole series there were 68 children with a caries figure below 0.74 and averaging 0.56 and sixty-eight with a caries figure above 1.24 and averaging 1.52. The respective increases in the average caries figure for the two groups was 0.28 and 0.44. Whether this is due to the influence of heredity, family diet or other factors, it is impossible to say. It is of interest to note that Hanke observed the same phenomenon among children on identical diets.

The caries figure of hypoplastic teeth showed no greater tendency to increase than that of teeth with no evidence of gross hypoplasia.

## SUMMARY AND CONCLUSIONS

An experiment was conducted to test the effect of fat-soluble vitamin concentrates upon the progress of dental caries

The concentrates were given in tablet form, the daily ration for each child containing not less than 6000 U.S.P. units of vitamin A and 1470 Steenbock units of vitamin D in the form of viosterol.

The daily ration was superimposed upon the ordinary diet of the child, no attempt being made to interfere with the food intake in any way.

The experimental group comprised 147 children of both sexes and of the average age of approximately 13 years at the beginning of the experimental period.

A comparable group of 171 controls receiving no vitamin concentrates was kept under observation.

The first and final inspections were made, respectively, in October, 1932, and December, 1933.

It was felt that children of this age would be ideal for such a study, in view of the fact that the caries process is most active around the age of puberty.

No beneficial effect could be observed in respect to the incidence of dental caries when the two groups were compared, as judged by 1) the average increase in percentage of affected teeth, 2) the average percentage increase in the number of cavities, or in the number of cavities per mouth, 3) the percentage increase in the average caries figure.

Furthermore, the percentage of teeth which erupted and became carious subsequent to the first examination was just as high in the group receiving concentrates as in the control group.

The caries process was equally as active between the second and third inspections as between the first and second.

One therefore feels justified in drawing the conclusion that without otherwise altering the diet, the administration of vitamins A and D to children of this age and within the period of this investigation, has no appreciable effect upon the rate of progress of the caries process in teeth already erupted or which erupt during the experimental period.

The necessity for radiographic surveys in all studies of this nature is evidenced by the data presented.

Of some interest is the demonstrated tendency for children showing an originally low caries incidence to maintain a lower absolute increase in the average caries figure than those with a high caries figure at the beginning of the experimental period.

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## LITERATURE CITED

- AGNEW, M. C., R. G. AGNEW, AND F. T. TISDALL 1933 The production and prevention of dental caries. *J. Am. Dent. Assn.*, vol. 20, pp. 193-212.
- BECKS, H., AND W. B. RYDER 1931 Experimental rickets and calcification of dentine. *Arch. Path.*, vol. 12, pp. 358-386.
- BLACKBERG, S. N., AND J. D. BERKE 1932 Effects of ante-natal and post-natal deficiency of vitamin D on animal dentition. *J. Dent. Res.*, vol. 12, p. 349.
- 1932 Dental caries experimentally produced in rats. *J. Dent. Res.*, vol. 12, p. 609.
- BLOCH (CITED BY K. U. TOVERUD AND G. TOVERUD) 1931 Studies on the mineral metabolism during pregnancy and lactation and its bearing on the disposition to rickets and dental caries. *Acta Paediatrica*, vol. 12, suppl. II, p. 80.
- BOYD, J. D., AND C. L. DRAIN 1930 Dietary control of dental caries. *J. Am. Dent. Assn.*, vol. 17, p. 740.
- BOYD, J. D., C. L. DRAIN, AND M. V. NELSON 1929 Dietary control of dental caries. *Am. J. Dis. Children*, vol. 38, pp. 721-725.
- BRODSKY, R. H., AND LUDWIG SCHOENTHAL 1933 Further studies on the etiology and control of dental caries. *J. Dent. Res.*, vol. 13, p. 227.
- BUNTING, R. W., E. DELVES, AND D. G. HARD 1930 The prevention of dental caries. *Proc. Soc. Exp. Biol. and Med.*, vol. 27, pp. 426-427.
- FRIEL, G., R. C. S. IREL, AND J. C. M. SHAW 1931 Some notes concerning dental caries in South African children. *Brit. Dent. J.*, vol. 52, pp. 309-320.
- HESS, A. F., AND H. ABRAMSON 1931 a The etiology of dental caries. *Dental Cosmos*, vol. 73, pp. 849-866.
- 1931 b The etiology of dental caries. *Dental Cosmos*, vol. 73, p. 849.
- HOFFERT, C. A., P. A. WEBBER, AND T. L. CANNIFF 1932 The production of dental caries in rats fed an adequate diet. *J. Dent. Res.*, vol. 12, p. 164.
- JONES, M. R., N. P. LARSEN, AND G. P. PRITCHARD 1930 Dental disease in Hawaii. *Dental Cosmos*, vol. 72, p. 699.
- KARSHAN, M. 1929 Calcification of teeth and bones on rachitic and non-rachitic diets. *Proc. Soc. Exp. Biol. and Med.*, vol. 27, pp. 200-202.
- KNOWLTON, F. D. 1930 The relation of diet to production of dental caries in young rats. *Proc. Soc. Exp. Biol. and Med.*, vol. 27, p. 757.
- KRONFELD, R., AND F. J. BARKER 1932 Experimental study of the influence of vitamin D on the hard tissues of white rats. *J. Am. Dent. Assn.*, vol. 19, pp. 105-128.
- LILLY, C. A. 1932 Failure to produce experimental dental caries in the white rat with high carbohydrate diet and *Bacillus acidophilus* or with vitamin D deficiency. *J. Nutrition*, vol. 5, pp. 175-181.
- MACKAY, H., AND S. F. ROSE 1931 Vitamin D deficiency, dental caries and tonsillar enlargement. *Lancet*, December 5, pp. 1230-1235.
- MARSHALL, J. A. 1927 Dental caries and pulp sequelae resulting from experimental diets. *J. Am. Dent. Assn.*, vol. 14, pp. 3-37.
- 1928 Control of dental caries by means of diet. *J. Am. Dent. Assn.*, vol. 15, p. 295.

- MCBEATH, E. C. 1933 Nutritional control of dental caries in children over 2-year period. *J. Dent. Res.*, vol. 13, p. 243.
- MELLANBY, MAY, C. L. PATTISON, AND J. W. PROUD 1924 The effect of diet on the development and extension of caries in the teeth of children. *Brit. Med. J.*, vol. 2, p. 354.
- 1926 Some factors of diet influencing the spread of caries in children. *Brit. Dent. J.*, vol. 47, p. 1045.
- 1928 The action of vitamin D in preventing the spread and promoting the arrest of caries in children. *Brit. Med. J.*, vol. 2, p. 1079.
- 1929 Diet and the teeth. Part I. *Brit. Med. Res. Council Report*, no. 140.
- 1930 Diet and the teeth. Part II. *Brit. Med. Res. Council Report*, no. 153.
- 1931 The influence of diet on caries in children's teeth. *Brit. Med. Res. Council Report*, no. 159, pp. 1-19.
- ORBAN, B. 1927 Nutrition and teeth. *J. Am. Dent. Assn.*, vol. 14, p. 1619.
- PRICE, WESTON A. 1932 The experimental basis for a new theory of dental caries with chemical procedures for determining immunity and susceptibility. *Dental Cosmos*, vol. 74, pp. 1139-1156.
- SHIBATA, MAKOTO 1931 Lack of vitamin D on development of the teeth. *Japan. Exp. Med.*, vol. 9, p. 21.
- TOVERUD, K. U., AND G. TOVERUD 1931 Studies on the mineral metabolism during pregnancy and lactation and its bearing on the disposition to rickets and dental caries. *Acta Paediatrica*, vol. 12, suppl. II, pp. 67-106.
- WIELAND, EMIL 1931 Rachitis und Rachitistherapie. *Schweiz. med. Wochenschr.*, Bd. 61, S. 25-29.
- WILSON, D. C., AND E. SURIE 1930 Osteomalacia. Studies I and IV. *Indian J. Med. Res.*, vol. 3, pp. 881-905.
- WOLBACH, S. B., AND P. R. HOWE 1933 The incisor teeth of albino rats and guinea pigs in vitamin A deficiency and repair. *Am. J. Path.*, vol. 9, pp. 275-293.

# THE USE OF THE METHOD OF PARTIAL REGRESSION IN THE ANALYSIS OF COMPARATIVE FEEDING TRIAL DATA.<sup>1</sup> PART II

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## INTRODUCTORY

In a previous paper (Crampton and Hopkins, '34) it was shown that the method of partial regression offers attractive possibilities as an aid to the interpretation of comparative feeding trial data. As a means of correcting live weight gains for effects of varying initial weights and feed consumption of pigs, it appears to be more satisfactory than the methods in common use. It was pointed out that the analysis might be made on the final weights of the animals equally well as on gains in weight, correcting them, as was done in the case of gains, by means of the partial regression formula for differences in initial weight and feed eaten, for, after all, the calculation of gains is but an attempt to correct for variations in initial weight. If all pigs of a trial were of the same weight at the start and consumed equal quantities of feed, a comparison of final weights would be the simplest analysis possible. It would seem, therefore, that if the effects of differences in the initial weights of the pigs and of differences in their feed consumption could be efficiently corrected for by partial regression, the final weights of the pigs would often be the logical data on which to base statistical analysis of such trials.

<sup>1</sup> Contribution from the Faculty of Agriculture of McGill University, Macdonald College, P. Q., Canada. Journal series no. 37.

The final weights of the four series of pigs referred to in the previous paper will, therefore, be treated in this way, in order to illustrate its possibilities, after which the application of the method to comparative trials involving several differently treated lots will be illustrated by the analysis of a further series of results, obtained in an actual experiment of this type.

#### ANALYSIS OF FINAL WEIGHTS

*Experimental data.* The experimental results used in this study were derived from the same source as those employed in the previous paper, and, excepting that in place of gains ( $y_1$ ), final weights ( $y_2$ ) were used, the analysis was performed by the same method.

The analysis of the variance and covariance of initial weight, feed consumption, and final weight is given in table 1.

It will be seen from table 1 that final weight is closely correlated with both initial weight and feed consumption. This is also true of gains, excepting in the D series, in which gains do not show a significant correlation with initial weight.

*Regression analysis.* The partial regression coefficients (computed as explained in part I of this series), of final weight on initial weight and feed consumption for the four series are given in table 2.

The standard errors of the various coefficients are also indicated, together with the ratio of the coefficients to their standard errors and the probability of obtaining such values by chance sampling from a population in which the attributes in question are uncorrelated.

It will be noted from table 2 that all  $b_2$  coefficients are highly significant, as are the  $b_1$  for series A, B, and C.

The regression of final weight on initial weight ( $b_1$ ) increases with increasing age of pig from 0.791 to 1.116. This means that in series A, a pig 10 pounds heavier than another at the outset will, on the average, be only 7.9 pounds heavier at the end of a 30-day feeding period, feed consumption being equal. In series C, on the other hand, differences in weight

at the outset are magnified 1.12 times at the end of the period, in the absence of differences in feed consumption.

TABLE 1

*Standard deviation of and correlation between initial weight, feed eaten, and final weight of swine of different age groups*

VARIABLES	MEAN	VARIANCE OR CO- VARIANCE	S.D.	C.V.	r	r NECESSARY FOR P = 0.01
Series A (N = 167)						
Initial weight	28.6	42.59	6.53	22.83	....	....
Feed eaten	106.5	408.66	20.22	18.99	....	....
Final weight	66.8	156.66	12.52	32.22	....	....
Initial weight $\times$ final weight ( $x_1 y_1$ )	....	66.08	....	....	0.81	0.25
Initial weight $\times$ feed ( $x_1 x_2$ )	....	80.39	....	....	0.61	0.25
Feed eaten $\times$ final weight ( $x_2 y_1$ )	....	228.30	....	....	0.90	0.25
Series B (N = 69)						
Initial weight	64.1	155.51	12.47	19.45	....	....
Feed eaten	391.7	2411.22	49.10	12.54	....	....
Final weight	158.7	493.48	22.21	13.99	....	....
Initial weight $\times$ final weight ( $x_1 y_1$ )	....	226.75	....	....	0.82	0.32
Initial weight $\times$ feed ( $x_1 x_2$ )	....	402.30	....	....	0.66	0.32
Feed eaten $\times$ final weight ( $x_2 y_1$ )	....	900.38	....	....	0.83	0.32
Series C (N = 89)						
Initial weight	155.5	471.40	21.71	13.96	....	....
Feed eaten	240.8	802.25	28.32	11.76	....	....
Final weight	206.7	758.41	27.54	13.32	....	....
Initial weight $\times$ final weight ( $x_1 y_1$ )	....	571.58	....	....	0.96	0.28
Initial weight $\times$ feed ( $x_1 x_2$ )	....	344.23	....	....	0.56	0.28
Feed eaten $\times$ final weight ( $x_2 y_1$ )	....	490.00	....	....	0.63	0.28
Series D (N = 48)						
Initial weight	67.6	129.30	11.37	16.81	....	....
Feed eaten	514.8	4564.75	67.56	13.12	....	....
Final weight	193.1	449.92	21.21	10.98	....	....
Initial weight $\times$ final weight ( $x_1 y_1$ )	....	165.85	....	....	0.69	0.37
Initial weight $\times$ feed ( $x_1 x_2$ )	....	575.41	....	....	0.75	0.37
Feed eaten $\times$ final weight ( $x_2 y_1$ )	....	1200.18	....	....	0.84	0.37



The increase in final weight per unit of feed eaten ( $b_2$ ) decreases markedly with increasing age of pig, from 0.40 pound weight for each additional pound of feed consumed in series A to 0.13 pound in series C.

*Reduction of variance.* As explained in section 3 of part I (Crampton and Hopkins, '34), the regression coefficients may be used to correct the observed final weights for variations

TABLE 2  
Partial regression coefficients of final weight on initial weight ( $b_1$ ) and feed eaten ( $b_2$ )

SERIES	N	INITIAL WEIGHT				FEED EATEN			
		$b_1$	Standard error ( $s \sqrt{C_{11}}$ )	$t = \frac{b}{s \sqrt{C_{11}}}$	P	$b_2$	Standard error ( $s \sqrt{C_{22}}$ )	$t = \frac{b}{s \sqrt{C_{22}}}$	P
A	167	0.791	0.0531	14.89	< 0.01	0.403	0.0177	22.77	< 0.01
B	69	0.866	0.1258	6.88	< 0.01	0.229	0.0319	7.17	< 0.01
C	89	1.116	0.2272	4.10	< 0.01	0.132	0.0340	3.88	< 0.01
D	48	0.257	0.2219	1.15	0.25	0.231	0.0374	6.17	< 0.01

TABLE 3  
Variance of final weights

SERIES	OBSERVED FINAL WEIGHTS				FINAL WEIGHTS CORRECTED BY REGRESSION FOR INITIAL WEIGHT AND FEED INTAKE				RELATIVE PRECISION
	D/F	Sum of squares	Variance	Standard error	D/F	Sum of squares	Variance	Standard error	
A	166	26004.9	156.66	12.52	164	2053.6	12.52	3.54	12.5
B	68	33556.5	493.48	22.21	66	6185.8	93.72	9.68	5.3
C	88	66740.4	758.41	27.54	86	4908.6	57.08	7.56	13.2
D	47	21146.2	449.92	21.21	45	6138.2	136.40	11.68	3.3

in initial weight and feed intake. In table 3 is given a summary of the variance of, a) observed final weights and b) final weights corrected by means of the partial regression coefficients for variations in the above factors. As might be expected, this correction results in a marked increase in precision.

Table 4, showing the variance of gains of the same animals, is included for comparison. It will be noted that the final

results are the same, whether gains or final weights form the basis of the analysis, the variance and standard deviations after correcting for effects of differences in initial weight and of feed intake being identical. If the actual weights are used, the labor of calculating the gains is avoided.

TABLE 4  
*Variance of gains*

SERIES	OBSERVED GAINS				GAINS CORRECTED BY REGRESSION FOR INITIAL WEIGHT AND FEED INTAKE				RELATIVE PRECISION
	D/F	Sum of squares	Variance	Standard error	D/F	Sum of squares	Variance	Standard error	
A	166	11134.6	67.08	8.19	164	2054.2	12.52	3.54	5.3
B	68	15468.2	227.47	15.08	66	6190.5	93.79	9.68	2.4
C	88	7625.2	86.65	9.31	86	4908.4	57.07	7.56	1.5
D	47	11634.1	247.53	15.73	45	6139.8	136.44	11.68	1.8

#### ANALYSIS OF RESULTS OF COMPARATIVE TRIALS

By means of the analysis of covariance (Fisher, '30, sec. 49, 1) it is possible to determine the regression of final weight on initial weight and feed consumption from the observed variance and covariance within the lots of a comparative trial. The regression coefficients may be employed as before to reduce the variance within lots, and also to make due allowance for the effects of differences in average initial weight and feed consumption between lots, thus enabling more accurate and unbiased conclusions to be drawn. To illustrate this application of the method, the results of a test recently completed at Macdonald College have been analyzed in this way and will be used as an example.

This trial involved five comparative lots of hogs fed over a period of 105 days. The pigs were allotted and started on test at 60 days of age. They were individually penned and hand full-fed throughout the trial. The comparative rations differed only in protein level, but the details of ration composition need not be given here, as it is the method of analysis rather than results of this particular trial which we wish to demonstrate.

Table 5 gives the initial and final weights of the pigs, the gain in live weight in 105 days of feeding, and the quantities of feed eaten during the test. The mean gain and final weight, actual and corrected, of each lot are also inserted.

The variance and covariance of the quantities listed in table 5 have in table 6a been analyzed into portions attributable to treatment differences, to differences between replicates, and to residual or error deviations. The required partial regression coefficients may now be estimated from the residual sums of squares and products, these representing the total covariance of initial weight, gain, etc., within lots after the elimination of treatment and replicate differences.

The partial regression coefficients of gain on initial weight ( $b_1$ ) and on feed intake ( $b_2$ ) thus calculated are:

$$b_1 = -0.01304; \quad b_2 = 0.24118$$

The corresponding coefficients using final weight instead of gain are:

$$b_1 = 0.98696; \quad b_2 = 0.24118$$

The analysis of variance of gains corrected for differences in initial weight and feed consumption by the regression formula is given in table 6b. The corrected sums of squares are obtained from the uncorrected values of table 6a by means of the relationship:

$$S(y - b_1 x_1 - b_2 x_2)^2 = S(y^2) - 2 b_1 S(x_1 y) - 2 b_2 S(x_2 y) + b_1 b_2 S(x_1 x_2) + b_1^2 S(x_1^2) + b_2^2 S(x_2^2)$$

( $x_1$ ,  $x_2$  and  $y$  being understood to be measured from their respective means), which is applied to each line of table 6a in turn. In the case of the residuals from which  $b_1$  and  $b_2$  were estimated, the right member reduces to

$$S(y^2) - b_1 S(x_1 y) - b_2 S(x_2 y)$$

Lot means are corrected for differences in average initial weight and feed consumption as follows:

Corrected mean =  $(\bar{Y}_1 - \bar{Y}) - b_1 (\bar{x}_{11} - \bar{x}_1) - b_2 (\bar{x}_{12} - \bar{x}_2) + \bar{Y}$  when  $\bar{Y}$  is the general mean (gain or initial weight),

$\bar{Y}_1, \bar{Y}_2, \dots, \bar{Y}_n$  the means of lots 1, 2, etc.,

$\bar{x}_{11}, \bar{x}_{12}, \dots, \bar{x}_{1n}$  the mean initial weight ( $x$ ) of lots 1, 2, etc.,

$\bar{x}_{21}, \bar{x}_{22}, \dots, \bar{x}_{2n}$  the mean feed eaten ( $x$ ) of lots 1, 2, etc., and

$b_1$  and  $b_2$  the regression coefficients of gain or final weight on initial weight and feed eaten, respectively.

TABLE 5  
Weights, gains, and feed consumption of pigs in comparative feeding trial

REPLI- CATE	LOT I					LOT II					LOT III					LOT IV					LOT V				
	Initial weight $x_1$	Feed eaten $x_2$	Gain $y_1$	Final weight $y_2$		Initial weight $x_1$	Feed eaten $x_2$	Gain $y_1$	Final weight $y_2$		Initial weight $x_1$	Feed eaten $x_2$	Gain $y_1$	Final weight $y_2$		Initial weight $x_1$	Feed eaten $x_2$	Gain $y_1$	Final weight $y_2$		Initial weight $x_1$	Feed eaten $x_2$	Gain $y_1$	Final weight $y_2$	
1	30	674	165	195		26	699	168	194		39	708	164	203		41	716	185	226		41	831	201	242	
2	21	628	156	177		24	626	180	204		34	614	156	190		35	769	195	230		36	754	189	225	
3	21	661	159	180		20	668	180	200		32	733	189	221		32	733	186	218		32	722	173	205	
4	33	694	167	200		35	668	166	201		35	663	138	173		34	742	201	235		35	728	193	228	
5	27	713	170	197		25	707	170	195		32	607	153	185		32	624	165	197		32	646	164	196	
6	24	585	146	170		26	651	161	187		35	745	190	225		35	710	175	210		36	678	160	196	
7	20	575	130	150		20	672	171	191		30	637	160	190		30	742	187	217		30	763	200	230	
8	29	638	151	180		31	660	169	200		29	662	172	201		28	648	177	205		28	625	142	170	
9	28	632	164	192		29	769	179	208		32	609	142	174		34	628	166	200		32	710	184	216	
10	26	637	158	184		27	666	191	218		25	596	155	180		26	601	165	191		26	651	149	175	
Means	25.9	643.7	156.6	182.5		26.3	678.6	173.5	199.8		32.3	657.4	161.9	194.2		32.7	691.3	180.2	212.9		32.8	710.8	175.5	208.3	
Cor- rected mean <sup>1</sup>			164.6	194.4				173.1	202.9				166.5	196.5				176.6	206.7				167.2	197.2	

<sup>1</sup> See table 7.

Tables 6a, 6b, and 6c give the analysis of variance of the gains and of the final weights, respectively, both before and after correction for the effects of varying initial weight and feed intake.

It will be noted that in the analysis of both tables, the variance between replicates, corresponding to 9 degrees of freedom, has been separated from the error sum of squares. In the present instance, this variance is actually less than the expectation from the residual variance within lots, with the result that the estimated standard error from the 36 residual

TABLE 6a  
*Variance and covariance of gains and final weights*

VARIANCE DUE TO	DEGREES FREEDOM	VARIANCE				COVARIANCE				
		Initial weights ( $x_1$ )	Feed eaten ( $x_2$ )	Gain ( $y_1$ )	Final weights ( $y_2$ )	$x_1 x_2$	$x_1 y$	$x_2 y$	$x_1 y_2$	$x_2 y_2$
Total	49	27.18	3149.95	288.05	380.29	149.76	32.53	766.15	59.71	915.90
Treatment	4	127.30	7101.23	976.63	1435.43	546.95	165.75	2352.18	293.05	2899.12
Replicate	9	50.49	3905.59	159.73	276.36	278.22	33.07	696.68	83.56	974.90
Error	36	10.23	2522.01	243.62	189.03	73.51	17.59	607.29	27.83	680.80

TABLE 6b

VARIANCE DUE TO	DEGREES FREEDOM	SUMS OF SQUARES OF GAINS		CORRECTED VARIANCE	1/2 log .	STANDARD ERROR
		Uncorrected gains	Corrected by regression			
Total	49	14114.4	4979.3	101.62		
Treatment	4	3906.5	1024.0	256.00	2.7725	
Replicate	9	1437.6	449.8	49.98		
Error	36 (34 <sup>1</sup> )	8770.3	3505.8	103.12	2.3179	10.15

TABLE 6c

VARIANCE DUE TO	DEGREES FREEDOM	SUMS OF SQUARES OF FINAL WEIGHTS		CORRECTED VARIANCE	1/2 log .	STANDARD ERROR
		Uncorrected final weights	Corrected by regression			
Total	49	18634.4	4980.5	101.64		
Treatment	4	5741.7	1024.1	256.00	2.7725	
Replicate	9	2487.2	450.0	50.00		
Error	36 (34 <sup>1</sup> )	10405.5	3506.2	103.12	2.3179	10.15

<sup>1</sup>Two additional degrees of freedom lost through calculation of two constants  $b_1$  and  $b_2$  from this data. Hence for error in corrected sums of squares the degrees of freedom is 34 instead of 36.

degrees of freedom in table 6a is 15.6 pounds, as compared with the value of 15.1 obtained from the total 45 degrees of freedom within lots. When significant differences between replicates do exist, however, a corresponding diminution of error is effected by the elimination of variation arising from this source. The reduction of the error variance in this way is quite legitimate, and is indeed one of the advantages to be derived from the structure of the experiment. Average differences between replicates affect all the members of a given replicate equally, and hence do not enter into treatment comparisons.

Comparison of the error variance of the uncorrected and corrected gains (or final weights) indicates the increase in

TABLE 7  
*Corrected mean gains and final weights from table 5*

LOT	MEAN GAIN	MEAN FINAL WEIGHT
I	164.6	194.4
II	173.1	202.9
III	166.5	196.5
IV	176.6	206.7
V	167.2	197.2

relative precision effected by the correction. In the case of the gains the ratio is 10 to 23, and in the case of final weights 10 to 28. In other words, equally reliable results could be obtained from ten pigs whose gains were corrected by this method for differences in feed intake and varying initial weights as from twenty-three pigs using uncorrected gains.

Attention has already been directed to the fact that the same estimate of error is arrived at whether gains or final weights are subjected to analysis. Table 7 illustrates a further point, namely, the identity of the differences between lots obtained in each case. This identity is a necessary consequence of the gain being a particular linear function (the difference) of the initial and final weights. The calculation of gains is thus seen to contribute no increase in accuracy to the analysis.

*The necessary difference.* a) Test of significance.<sup>1</sup> Using the standard error of 10.15, the difference between two lots, of ten pigs each, necessary to indicate that treatment had a significant effect will be:

$$\frac{s\sqrt{2}}{\sigma_n} \times t_{P=.05} = \frac{10.15 \times 1.414}{8.16} \times 2.262 = 10.3 \text{ lb.}$$

b) Estimation of effects of treatment. Between means showing this (10.3 lb.) or larger differences the necessary difference to cover experimental error, with odds of 19 to 1, will be—

$$\frac{10.15 \times 1.141}{8.16} \times 1.833 = 8.3 \text{ lb.}$$

since for odds of 19 to 1,  $t$  for  $P = 0.1$  may now be used instead of  $t$  for  $P = 0.05$ .

Examination of the corrected means indicates a significant difference between lots IV and I in favor of lot IV. Deducting from the difference of 12.0 between these two means the necessary difference of 8.3 leaves a net difference in gains creditable to treatment of 3.7 pounds. We should be justified, therefore, in claiming just over 2 per cent faster gains due to treatment in lot IV as compared to lot I.

#### SUMMARY

A previous statistical study of the relationship between the initial weight and feed consumption of swine of four different age groups and their performance in experimental feeding trials has been extended, and further examples of the application of these relationships to the reduction of the experimental error in such trials have been given.

In all age groups a highly significant relationship between final weight and feed eaten is demonstrable, the increase in final weight per unit of additional feed consumed decreasing

<sup>1</sup>It has recently been shown by Cochran, (Proc. Camb. Phil. Soc., XXX, 177-191) that the ratio of the corrected 'treatment' to 'error' sum of squares will not follow exactly the Z distribution. If, therefore, it is also desired to apply the Z test of significance to the observations as a whole, a 'treatment' sum of squares must be computed by a slightly different method.

with advancing age of pig. The final weight attained is also influenced by the initial weight of the pig, except possibly in the case of young pigs fed through to market weight.

It is shown that identical results are obtained whether the gains or final weights attained are corrected for variations in feed consumption by means of the appropriate regression formula. The usual calculation of gains thus leads to no increase in accuracy, and unless of particular interest may be omitted.

The application of the method of partial regression to the analysis of comparative feeding trial data has been illustrated, using the results of an experiment recently completed at Macdonald College in which five lots of ten pigs each were fed for a period of 105 days on rations differing in protein level.

#### LITERATURE CITED

- CRAMPTON, E. W., AND J. W. HOPKINS 1934 The use of the method of partial regression in the analysis of comparative feeding trial data. Part I. *J. Nutrition*, vol. 8, pp. 113-124.
- FISHER, R. A. 1930 *Statistical methods for research workers*. 3rd ed.





# RESULTS OF THE INGESTION OF COD LIVER OIL AND YEAST ON CALCIUM AND PHOSPHORUS METABOLISM OF WOMEN

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It is generally taken for granted that the normal adult, living in the usual urban environment with little exposure to sunshine, needs a dietary enriched with vitamin D for the regulation of calcium and phosphorus metabolism, although the proof of this supposition has not been demonstrated through the administration of cod liver oil (Hart, Tourtellotte and Heyl, '28), irradiated ergosterol (Bauer, Marble and Claffin, '32), or cod liver oil and direct irradiation of the subjects with the mercury vapor lamp (Dye, '30). Moreover, yeast with its potent source of the vitamin B complex is alleged to benefit mineral metabolism in the adult (Bogart and Trail, '22).

In view of the apparent confusion concerning the effectiveness of vitamins upon the calcium and phosphorus retention in the adult, additional information has been obtained upon the influence of cod liver oil alone and of cod liver oil and yeast on the calcium and phosphorus metabolism of three healthy young women. These women were under observation for 38 days, during which they consumed their customary diets, unrestricted in quality and quantity, and carried on their usual daily activities, thus reproducing the conditions existing under the ordinary adult business life. The standardized metabolic technic used in this laboratory was followed for weighing and sampling the diets for analyses and for quantitatively collect-

ing, preparing, and analyzing the excreta (Macy, Hunscher, Nims and McCosh, '30; Donelson, Nims Hunscher, Shukers and Macy, '31).

The initial control period consisted of a 15-day pre-experimental period divided into five 3-day metabolic balances when no changes were made in the dietary. Following immediately thereafter, 15 gm. of cod liver oil were given daily, and, after an adjustment period of 11 days, four successive 3-day experimental metabolic balances were made, during the first two of which the influence of cod liver oil alone on calcium and phosphorus metabolism was observed, and during the last two periods the effect of 10 gm. of yeast daily in addition to the cod liver oil<sup>1</sup> was observed.

Although subjects X, Y, and Z were taking a liberal daily diet, containing an average of 0.819, 1.094, and 1.055 gm. of calcium, respectively, 70 per cent of which was secured in the form of milk and cheese, they gave individual responses to the same diet, and during the first five metabolic balances in the pre-experimental period, X and Y were practically in equilibrium, while Z was losing on an average of 0.194 gm. of calcium per day (table 1). There were noticeable variations among the subjects in the retentions of calcium which were not always commensurate with changes in the level of intake. The administration of cod liver oil did not stimulate any definite change in metabolism, for the retentions of calcium were as variable and in approximately the same range of magnitude as they were before the supplement was made to the diet, except in the case of subject X, who showed some improvement. These negative responses of the calcium balance in adults to the administration of cod liver oil corroborate the findings of other investigators (Hart, Tourtellotte and Heyl, '28; Bauer, Marble and Claffin, '32; Dye, '30).

The fat content of the diet and the calcium balances manifest no relationship, for, in some instances, particularly in the

<sup>1</sup> We wish to express our thanks and appreciation to A. D. Emmett, Ph.D., and E. A. Sharp, M. D., of Parke, Davis & Co., Detroit, for the cod liver oil, and to Maurice H. Givens, Ph.D., of the Northwestern Yeast Company, Chicago, for the yeast used in this study.



results of X and Y, an increase of fat in the diet was accompanied by an increase in the per cent of total outgo of calcium in the feces, whereas this was not always the rule. These observations are in accord with those of Mallon, Jordon and Johnson ('30), who concluded that "fat per se cannot be said to exercise a definite influence upon the calcium retention" in women.

The diets contained an average of 1.394, 1.543 and 1.631 gm. of phosphorus per day for X, Y, and Z, respectively (table 2). During the control period the resultant mean phosphorus retentions were + 0.299, and + 0.208, and + 0.225 gm., but after cod liver oil therapy the average retentions changed to + 0.034, + 0.420, and + 0.224 gm., and during the simultaneous administration of yeast and cod liver oil they were -0.260, + 0.270, and -0.260 gm. for subjects X, Y, and Z, respectively. Thus it is seen that after cod liver oil alone was given there was a decrease in the phosphorus retention for the subject X, an increased retention for subject Y, and no change for subject Z. On the other hand, when cod liver oil and yeast were given together, there was a lowered phosphorus retention in all three subjects. These negative findings, resulting from the addition of cod liver oil, corroborate those of Bauer, Marble, and Claffin ('32), but are in contrast to those observed by Dye ('30). When cod liver oil was included in the diet, the calcium and phosphorus increased in the urine and decreased in the feces of subjects X and Y.

From the results obtained in twenty-seven 3-day calcium and phosphorus balances on three healthy women each of whom was under observation for 38 continuous experimental days when consuming her usual unrestricted diet and participating in her customary activities, no consistent physiologic changes were noted in the retention of calcium and phosphorus due to either cod liver oil or yeast.

TABLE 2

## Daily phosphorus balance

PERIODS	SUBJECT X						SUBJECT Y						SUBJECT Z					
	Intake			Outgo			Intake			Outgo			Intake			Outgo		
				Total	Urine	Feces				Balance	Total	Urine				Feces	Balance	Total
	gm.	per cent	per cent				gm.	per cent	per cent				gm.	per cent	per cent			
I	1.091	1.157	37.4	62.6	-0.066	1.175	1.047	55.5	44.5	+0.128	1.203	0.952	47.5	52.5	+0.251			
II	1.484	0.890	42.5	57.5	+0.594	1.577	1.421	50.4	49.6	+0.156	1.581	1.412	38.0	62.0	+0.169			
III	1.382	1.057	48.4	51.6	+0.325	1.838	1.501	41.3	58.7	+0.337	1.936	1.668	42.1	57.9	+0.268			
IV	1.382	1.197	36.7	63.3	+0.185	1.582	1.371	51.1	48.9	+0.211	1.675	1.573	53.1	46.9	+0.102			
V	1.629	1.174	36.8	63.2	+0.455	1.543	1.335	49.6	50.4	+0.208	1.631	1.423	42.4	57.6	+0.337			
Average	1.394	1.095	40.4	59.6	+0.299	1.543	1.335	49.6	50.4	+0.208	1.631	1.406	44.6	55.4	+0.295			
Experimental—cod liver oil																		
I	1.213	1.146	59.5	40.5	+0.067	1.650	1.039	53.7	46.3	+0.611	1.575	1.701	43.6	56.4	-0.126			
II	1.675	1.671	42.6	57.4	+0.004	1.280	1.050	50.7	49.3	+0.230	2.224	1.651	42.7	57.3	+0.573			
Average	1.444	1.408	51.0	49.0	+0.036	1.465	1.044	52.2	47.8	+0.420	1.900	1.676	43.2	56.8	+0.224			
Experimental—cod liver oil and yeast																		
I	1.638	1.700	51.1	48.9	-0.062	2.004	1.734	39.9	60.1	+0.270	2.080	1.803	59.7	40.3	+0.277			
II	1.084	1.541	61.7	38.3	-0.457						1.507	2.305	45.2	54.8	-0.798			
Average	1.361	1.621	56.4	43.6	-0.260						1.794	2.054	52.4	47.6	-0.260			

## LITERATURE CITED

- BAUER, W., A. MARBLE, AND D. CLAFLIN 1932 Studies on the mode of action of irradiated ergosterol I. Its effect on the calcium, phosphorus, and nitrogen metabolism of normal individuals. *J. Clin. Invest.* vol. 11, p. 1.
- BOGETT, L. G., AND R. K. TRAIL 1922 Studies in inorganic metabolism. III. The influence of yeast and butter fat upon calcium assimilation. *J. Biol. Chem.*, vol. 54, p. 387.
- DONELSON, E., B. NIMS, H. A. HUNSCHER, C. F. SHUKERS, AND I. G. MACY 1931 Simple methods for metabolic balance studies and their interpretation. *J. Home Econ.* vol. 23, p. 267.
- DYE, M., 1930 Effect of the antirachitic factor and ultra-violet irradiation on calcium metabolism of women. Michigan State College Agricultural Experiment Station Report, June, p. 37.
- HART, M. C., D. TOURTELLOTTE, AND F. W. HEYL 1928 The effect of irradiation and cod liver oil on the calcium balance in the adult human. *J. Biol. Chem.*, vol. 76, p. 143.
- MACY, I. G., H. A. HUNSCHER, B. NIMS, AND S. S. MCCOSH 1930 Metabolism of women during the reproductive cycle. I. Calcium and phosphorus utilization in pregnancy. *J. Biol. Chem.*, vol. 86, p. 17.
- MALLON, M. G., R. JORDON, AND M. JOHNSON 1930 A note on the calcium retention on a high and low fat diet. *J. Biol. Chem.*, vol. 88, p. 163.

# THE DETERMINATION OF VITAMIN A VALUES BY A METHOD OF SINGLE FEEDINGS

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TWO FIGURES

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Establishment of the position of carotene as precursor of vitamin A does not imply that there is a sole precursor nor that problems of the formation, bodily storage and utilization of vitamin A can be dealt with in terms of only two substances, one the precursor and the other the vitamin itself. In fact, three carotenes (not necessarily of equal potency as vitamin A precursors) are already recognized, and one should also take account of the possibility that the carotenes are not the only substances from which vitamin A can be formed.

Hence, while much is being learned from experiments in which carotene and vitamin A are estimated separately by colorimetric methods and the spectrophotometric examination of the intensity of absorption bands (e.g., Moore, '30; Coward, Dyer, Morton and Gaddum, '31), there is still need for the measurement of vitamin A values which may be due in part to the vitamin itself, in part to one or more of the carotenes, and possibly also in part to other precursors. Such measurements must remain fundamental to the studies of the problems of specificity of vitamin A reactions and of the functioning of the precursors of vitamin A; and upon such studies the further development of our knowledge of this vitamin and its nutritional significance will doubtless largely depend.



Methods now current involve the feeding of the material under investigation continuously for 3 or more weeks. This not only calls for a large expenditure of the time and attention of the investigator but also implies the assumption, which must often be doubtful and sometimes improbable, that the material under investigation will remain unchanged throughout the weeks of such a feeding period.

Such studies, for example, as of autopsy material, of the tissues of individual experimental animals, of plants or their products at definite stages of growth or ripening, or of the freshly prepared products of laboratory investigation (whether synthetic or by fractionation of natural materials) would not only be greatly facilitated but would gain in scientific value if the material to be tested could be fed promptly when ready without the necessity of an assumption that the sample in hand is stable or that the next sample will exactly duplicate it and will be ready exactly when wanted. Samples only occasionally available may have much scientific value for the study of certain specific problems.

Cammack ('25) suggested that the continuous feeding process might be replaced by using a single feeding of vitamin A containing food to experimental rats and then measuring in days the increased survival of such animals over the negative controls. Nelson, Walker and Jones ('31, '32) have reported the use of single feedings of cod liver oil to rats and the consequent gain in survival as a means of studying the ability of the animal to store vitamin A.

If a method of single feedings were shown to be practical, it would be useful in the study of foods or other materials which are available in the fresh state for a very limited time, or which are obtainable only in small quantities.

The present work is an attempt to develop such a method. As a basis of this study, carotene, which been proposed as an international unit of reference, was used. Other materials were tested in parallel with carotene and the data obtained were examined in terms of the results of carotene feeding.

## EXPERIMENTAL RESULTS AND DISCUSSION

Albino rats of known nutritional history weighing 35 to 55 gm. at 21 to 28 days of age were used. They were depleted of their surplus body stores of vitamin A while maintained on vitamin A-free basal diet (Sherman and Munsell, '25); and the average weight of 162 rats at the end of the depletion period was 101 gm. The depletion time averaged 32.3 days, with 28 and 36 days as the extremes. The general experimental technic was that of Sherman and Burtis ('28) and Sherman and Batchelder ('31). The basal diet was: purified casein, 18 per cent; dried yeast, 10 per cent; Osborne and Mendel salt mixture, 4 per cent; sodium chloride, 1 per cent; cornstarch, 67 per cent. Vitamin D was incorporated in the diet in the form of viosterol in such an amount as to give antirachitic activity equivalent to the presence in the basal diet of 3 per cent of a cod liver oil having 100 vitamin D units per gram.

*Carotene* (m.p. 172° uncor.) was obtained from the British Drug Houses, Limited. It was administered orally in peanut oil solution (the peanut oil was shown by feeding tests to be inactive) using a glass syringe graduated in one hundredths of a cubic centimeter. The carotene solution was stored in a dark bottle at  $0^{\circ} \pm 1^{\circ}\text{C}.$ , and colorimetric readings, using potassium dichromate solution as a reference, were made frequently to check any change in color, which would indicate a change in potency of the carotene.

At the end of the depletion period, the experimental rats were divided into groups of comparable weight, sex, and litter; one group served as negative controls, and others received a single feeding of carotene or other material. Weekly weighings of all rats were made during the experimental period which lasted from the time when the single feeding was given until death.

Carotene was given in single feedings of 28  $\gamma$ , 56  $\gamma$ , 112  $\gamma$  ( $\gamma = 0.001$  mg.) and the growth responses and survival period are shown in figure 1. Broken lines indicate the death of one or more animals and the end point of each curve represents the average weight and survival in days for that group

of rats. It is observed that a large single feeding of carotene causes increased growth and survival over that obtained by the negative controls, and increasing the quantity of the single feeding gives further increases in growth and survival. The survival periods were: negative controls  $19.2 \pm 0.6$  days; after feeding 28  $\gamma$  carotene  $25.3 \pm 0.6$  days; after 56  $\gamma$  carotene  $31.7 \pm 0.7$  days; and after 112  $\gamma$  carotene  $38.1 \pm 1.4$  days. The increase in height of the curves is directly influenced by the amount of carotene fed and if the highest point on each curve is taken as the positive weight increase and divided by the quantity of carotene for that curve, the following results are obtained: 0.25 gm., 0.29 gm. and 0.22 gm. per  $\gamma$  carotene fed for the respective growth curves in figure 1. Thus within these limits growth is roughly, but not exactly, pro-

TABLE 1

*Areas under the growth curves of rats receiving single feedings of carotene, when measured from the extreme ends of the average weight curves*

AMOUNT OF CAROTENE FED	NUMBER OF ANIMALS USED	AREA IN SQUARE CENTIMETERS
28 $\gamma$	30	25
56 $\gamma$	21	50
112 $\gamma$	6	80

portional to the amount of carotene given in a single feeding; and in order to estimate as quantitatively as possible the vitamin A value of any substance by the single feeding method both the growth increase and the increase in time of survival should be taken into consideration. Measurement of the area under each curve apparently takes due account of both these factors.

In our first interpretation, such measurements were made using the negative control curve to bound one side of the area. A straight line drawn from the end of the carotene curve to the end of the negative control curve completed the area. Curves were plotted on cross section paper, of  $\frac{1}{10}$ -inch squares, each representing 1 gm. on the ordinates and 1 day on the abscissa line. A planimeter was used for measuring the areas which are recorded in table 1.

The ultimate loss in weight by the experimental animals is likely to be a variable factor, particularly as it may be much accentuated in the last day or two before death, and variation in the length of the curve due to this may cause an undue variation in the area measured.

In our final interpretation it has seemed to us better to avoid the introduction of such errors by measuring the areas shown

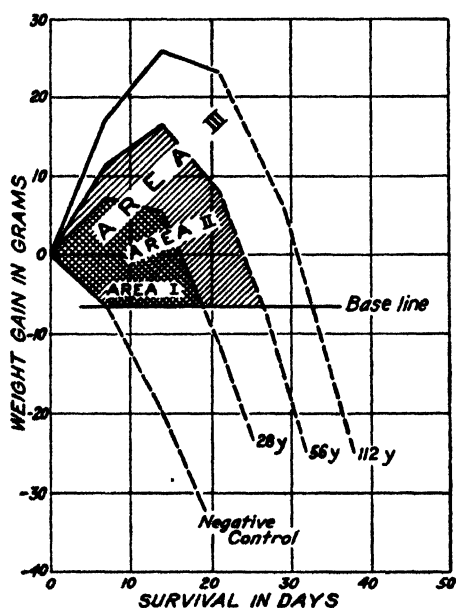


Fig. 1 Average gain curves showing how the areas were measured in securing the data shown in table 2. Shaded portion marked area I represents the area for the 28  $\gamma$  carotene feeding, area II for 56  $\gamma$  carotene, and area III for 112  $\gamma$  carotene. The 'base line' is drawn horizontally through the point representing the average weight-loss of the negative controls at the end of the first week.

by the shaded portion in figure 1. A horizontal line is drawn through the point representing the end of the first week of the negative control curve, which includes records of all the negative control animals, and this is used as the base line for all areas. Areas as thus measured are given in table 2.

Within the limits of these experiments, therefore, the data of table 2 show that doubling the amount of carotene fed approximately doubles the area under the curve.

*Cod liver oil* diluted with vitamin A-free peanut oil was given in single feedings to litter mates of the rats of the carotene series described above. A fresh solution was made every 2 weeks. The average survival period for twenty-five rats receiving 7 mg. cod liver oil was  $25.0 \pm 0.6$  days, while eighteen rats after receiving 14 mg. cod liver oil survived an average of  $29.9 \pm 1.1$  days. The rats on 7 mg. cod liver oil survived the same length of time as those on 28  $\gamma$  carotene, but figure 2 shows that the growth increase was less in the case of the cod liver oil. Measurements of the areas under the cod liver oil feeding curves were 6 and 12 sq.cm., respectively, and were thus in direct relation to the amounts of cod liver oil fed. The area obtained from the 14-mg. feeding was almost exactly

TABLE 2

*Areas under the growth curves of rats receiving single feedings of carotene. when measured with reference to the base line explained in the text*

AMOUNT OF CAROTENE FED	AREA IN SQUARE CENTIMETERS
28 $\gamma$	11
56 $\gamma$	25
112 $\gamma$	48

the same as for 28  $\gamma$  carotene, indicating that 14 mg. of the cod liver oil were equivalent to 28 units of the carotene here used and to 28 units of vitamin A as here found by the single feeding method.

Litter mates fed daily amounts of cod liver oil for a period of 5 weeks gave the following data: eight rats receiving 1.0 mg. cod liver oil daily averaged 30.4 gm. of gain; two rats receiving 0.5 mg. cod liver oil daily averaged 19.5 gm. of gain. One unit of vitamin is seen to be contained in 0.5 mg. cod liver oil and therefore 14 mg. contain 28 units of vitamin A as found by the feeding method hitherto used. The findings by the single feeding method are thus in agreement with those of the method of continuous feedings through 4 weeks or more.

*Kale* purchased on the open market during March, April and May, and of the dark green curly leaved variety, was

given in the fresh state in single feedings to litter mates of the rats of the carotene series. Results are shown in figure 2; for eight rats receiving a single feeding each of 0.7 gm. kale, the average area under the curve was 65 sq.cm.; and for nine rats receiving 1.4 gm. kale, 136 sq.cm.

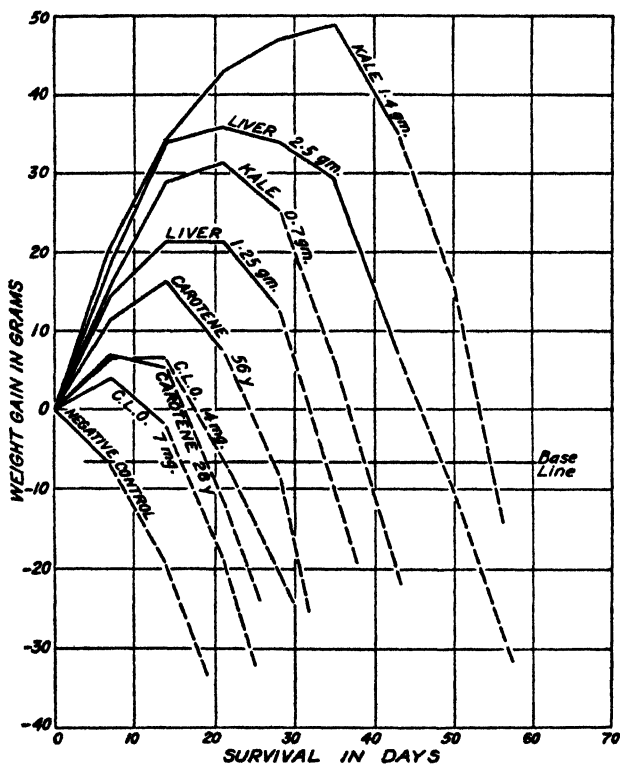


Fig. 2 Average gain curves for all animals on single feedings of different foods. (See also fig. 1.)

Using as a standard of reference the carotene curve of 25 sq.cm. which corresponds to 56 units, then 65 sq.cm. would be obtained by feeding 146 units of vitamin A, and 136 sq.cm. from feeding 305 units.

A group of rats received daily feeding of kale for 5 weeks and made the following average gains: six rats receiving 11 mg. daily of kale gained an average of 24 gm., and three rats receiving 5 mg. daily gained 12 gm. over that period.

The amount containing one unit of vitamin A is taken from this as 5 mg., therefore the single feedings of 0.7 gm. and 1.4 gm. by this method are seen to be equivalent to 140 and 280 units respectively. Thus the divergence from the older method shown by the new single feeding method was in this case 4 per cent for the lower level of feeding and 9 per cent for the higher level.

*Calf liver* used in single feedings of 1.25 gm. (three rats) and 2.5 gm. (four rats) gave growth and survival curves similar in shape to those obtained with carotene and areas were 41 sq.cm. and 83 sq.cm., respectively. Interpreted by means of the carotene curve of 25 sq.cm., these feedings of calf liver are equivalent to 91 units of vitamin A and 186 units. Thus there was good agreement of results at the two levels and by the single feeding test, the given sample of calf liver appears to have contained 73 units of vitamin A per gram.

Figure 2 summarizes the data obtained using the single feeding method for different test substances.

*Some factors influencing single feeding results.* Our carotene feeding experiments were fairly evenly distributed over a period of more than 1 year, so that any possible influence of season would be neutralized. The influence of sex was studied and the females were found to make smaller gains than the males; nine females receiving 56  $\gamma$  carotene gave an average area of 17 sq.cm. under the growth curve, and twelve males a corresponding area of 28 sq.cm. The data used throughout this paper are averages for both sexes which were almost equally divided on each level of feeding.

Without attempting any general statement of the efficiency with which the animal organism is able to absorb and utilize large amounts of carotene, it may be pointed out that the responses of the experimental animals are similar (fig. 2) and approximate a quantitative relationship to the amount of material fed, whether this supplied the vitamin A itself or its precursor. Thus carotene represents the purified and concentrated form of a precursor of vitamin A. Cod liver oil contains vitamin A as such. Kale contains carotene in its

natural combined or admixed state. Calf liver owes its potency in part to vitamin A, some of which has been formed from the carotene of the green feed of the calf, some of which has been received directly from the milk of the calf's diet; while possibly there is also some unchanged carotene in the liver. Yet the method here developed appears applicable to all of these four types of material.

Such findings indicate that the single feeding method is practicable for determining the vitamin A values of widely varying types of food material if the substance to be evaluated is fed in parallel with carotene, or standardized cod liver oil, and if the sexes are evenly distributed on the different levels of feeding.

Applying the single feeding method in this way to kale shows it to have a vitamin A value of 200 units per gram of fresh moist kale. This value is comparable with that previously found for escarole, and considerably higher than any of the values which we have seen reported for spinach.

#### SUMMARY

Standardized rats depleted of their surplus body stores of vitamin A were given single feedings of carotene, of cod liver oil, of kale, or of calf liver. The weight curves throughout the period of survival after such feedings were charted on a fixed scale and the areas bounded by the resulting curves were measured with a planimeter. The lower boundary of such an area may be established either by drawing a line between the extreme ends of the average curves concerned or by the use of a horizontal base line through a point found from the performance of all of the negative controls as explained in the text. The latter mode of computation is here preferred.

Within limits amply sufficient for experimental work, increasing the amount of vitamin A or precursor given in a single feeding gave an approximately proportional increase in the area under the curve.

Carotene curves were used as a standard of reference and the curves obtained by feeding cod liver oil, kale, and calf



liver were interpreted quantitatively in terms of this standard. Direct comparison showed that the method of single feedings results in data which can be evaluated in terms of the same units and with findings in close agreement with those of the methods of feeding through periods of 4 or more weeks as hitherto regarded most accurate and conclusive. The same method can be used with a standardized sample of cod liver oil (or any other suitable material) as a basis of reference.

The area of the growth and survival curves for males was greater than for females. When both sexes are used they should be used in equal numbers (or other fixed proportion) throughout.

The single feeding method yielded an average finding of about 200 units of vitamin A value per gram of fresh moist kale, thus indicating kale to be among the richest of known natural sources.

#### LITERATURE CITED

- CAMMACK, M. L. 1925 A quantitative study of the storage of vitamin A. Dissertation, Columbia University.
- COWARD, K. H., F. J. DYER, R. A. MOERTON, AND J. H. GADDUM 1931 The determination of vitamin A in cod liver oils, a) biologically, b) chemically, c) physically with a statistical examination of the results. *Biochem. J.*, vol. 25, p. 1102.
- MOORE, T. 1930 Vitamin A and carotene. *Biochem. J.*, vol. 24, p. 692.
- NELSON, E. M., R. WALKER, AND D. B. JONES 1931 The determination of vitamin A by a preventive method. *J. Biol. Chem.*, vol. 92, p. vi.
- 1932 The rate of transfer of ingested vitamin A to the liver of the rat. *J. Biol. Chem.*, vol. 97, p. vi.
- SHERMAN, H. C., AND E. L. BATCHELDER 1931 Further investigation of quantitative measurement of vitamin A values. *J. Biol. Chem.*, vol. 91, p. 505.
- SHERMAN, H. C., AND M. P. BURTIS 1928 Factors affecting the accuracy of the quantitative determination of vitamin A. *J. Biol. Chem.*, vol. 78, p. 671.
- SHERMAN, H. C., AND H. MUNSELL 1925 The quantitative determination of vitamin A. *J. Am. Chem. Soc.*, vol. 47, p. 1639.

## A VITAMIN B DEFICIENT RATION

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### ONE CHART

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Various substances have been used as a source of vitamin G in vitamin B-deficient rations. While autoclaved yeast or yeast concentrates have been most commonly employed, egg albumin (Chick and Roscoe, '29), special vitamin G concentrates (Guerrant and Dutcher, '32; and Roscoe, '30), and autoclaved meat (Sure, '33) have also been used. Milk is known to be relatively high in vitamin G and low in vitamin B. The recent work of Kuhn et al. ('33) and Booher ('33) indicates that highly potent vitamin G concentrates may be prepared from milk serum. In view of experiences at this laboratory (Bender, '32), as well as those reported from other sources, it seemed desirable to determine the merits of autoclaved dry milk and dry whey as sources of vitamin G for routine vitamin B assays. Although autoclaved dry whey has given uniform and consistently satisfactory results no detailed and comparative data have been reported. Such data are recorded in the present paper in comparison with the results obtained from autoclaved yeast and autoclaved milks.

### EXPERIMENTAL

Dry whey, commercial dry skim milk as prepared by the Roller Process, and fluid skim milk were autoclaved for varying periods of time, with and without the addition of water as a moistening agent for the dry products. Sodium hydroxide solution, sodium bicarbonate, or hydrated lime were added to

the dry products prior to autoclaving to give a predetermined degree of alkalinity. The dry skim milk and dry whey samples were autoclaved at 120°C. for 1-, 2-, and 5-hour periods. Decomposition of the dry products as indicated by color and odor was minimized by moistening irrespective of the period of heating. The liquid skim milk sample autoclaved for 1 hour only was found to have a decreased pH and was adjusted to the pH of the original milk before drying. The 2-hour sample was autoclaved for 1 hour, its pH adjusted to that of the original milk, autoclaved for another hour and its pH again adjusted to that of the original milk. These two samples and a sample of the original milk were dried by the double roller process. Three samples of yeast were treated similar to the dry whey and dry skim milk, except that the period of heating for all such samples was 5 hours. One sample was a pure dry yeast, another was dehydrated yeast obtained from a different source, and the third was a sample of fresh baker's yeast. After autoclaving, all these products were air dried or oven dried at temperatures not exceeding 100°C. The pH value of the test substances was determined before and after autoclaving with but few exceptions. In those instances where sodium bicarbonate or hydrated lime was added before autoclaving sufficient amounts of these alkaline substances were used to bring the reaction to pH 7.5. The pH value of the original materials before the addition of alkaline substances and before autoclaving, varied between 5.8 and 6.4. After autoclaving, the pH of all samples had decreased to between 4.4 and 5.8 with the exception of two yeast samples which showed a pH value of 8.8. The reaction of the products before autoclaving did not appear to materially influence the amount of vitamin B remaining after the treatment.

The amount of residual vitamin B present in the autoclaved substances was determined by feeding 15 parts of the autoclaved material in place of the carbohydrate of the basal ration. The rate of growth and period of time before cessation of growth were used as the criteria for judging the

amount of vitamin B remaining in the product. The basal ration was as follows: vitamin free casein,<sup>1</sup> 20 parts; agar-agar, 2 parts; Steenbock salt mixture,<sup>2</sup> 4 parts; dextrin or sucrose, 62 parts; Crisco, 10 parts; and cod liver oil, 2 parts.

In order to determine whether an adequacy of vitamin G was carried by the autoclaved products, varying amounts of rice polish (for these particular determinations as high as 200 mg. per day) were fed as daily supplements following cessation of growth. It has previously been shown by Supplee, Kahlenberg and Flanigan ('31) that rice polish in this amount or less, supplies an adequacy of vitamin B for rapid growth when sufficient vitamin G is present. When good growth resulted after supplementation with the rice polish, it was concluded that the autoclaved product contained sufficient vitamin G.

The experiments were carried out with white rats which were housed in individual metal cages with screened bottoms (2 mesh to the inch). The animals selected for the experiments weighed 40 to 50 gm. and were 20 to 24 days old. With very few exceptions, an equal number of both sexes were used in each test group. The McCollum type feed dish was used.

The data summarized in table 1 are presented for comparative purposes to show the character of the results obtained from the different periods of heat treatment to which the various products had been subjected. The data show that the vitamin B of the autoclaved dry whey appears to be destroyed. That the dry whey still retains sufficient vitamin G for growth is illustrated by typical curves in chart 1. When vitamin B was supplied by 200 mg. rice polish fed daily in the absence of vitamin G, or when vitamin G was supplied by 15 per cent autoclaved dry whey in the absence of vitamin B growth did not result; however, when the two products were combined, excellent growth was obtained. These curves are typical of the results obtained. The results from the animals

<sup>1</sup> The vitamin free casein is distributed by the Casein Manufacturing Company of America, New York.

<sup>2</sup> Steenbock, H., and E. M. Nelson. J. Biol. Chem., 1923, vol. 56, p. 362.

TABLE 1  
Effect on growth results of feeding with the basal ration 15 per cent autoclaved yeast, skim milk or whey products

GROUP	SAMPLE	AUTOCLAVE TREATMENT (120° C.)	NUMBER OF RATS STARTED	AVERAGE WEIGHT AT START	AVERAGE GRAMS GAIN PER WEEK FOR					
					First	Second	Third	Fourth	Fifth	Sixth
1	Yeast 2 <sup>1</sup>	Not autoclaved	4	46	29	31	22	14	14	6
2	Yeast 2	5 hours	32	46	23	15	10	6	3	2
3	Yeast 3 <sup>2</sup>	Not autoclaved	4	44	30	33	27	15	16	15
4	Yeast 3	5 hours	32	46	18	16	8	7	10	5
5	Yeast 4 <sup>3</sup>	5 hours	50	43	21	12	7	7	7	7
6	Dry skim milk	Not autoclaved	4	47	18	15	15	7	6	5
7	Dry skim milk	1 hour	16	45	9	10	6	3	1	2
8	Dry skim milk	2 hours	16	44	8	7	7	1	1	2
9	Dry skim milk	5 hours	16	45	8	6	5	-3	-1	0
10	Liquid skim milk	Not autoclaved but dried	4	41	17	13	14	5	3	4
11	Liquid skim milk	Autoclaved 1 hour and dried	8	42	12	12	3	-3	-7	0
12	Liquid skim milk	Autoclaved 2 hours and dried	4	41	12	9	5	-4	-6	-1
13	Dry whey	Not autoclaved	4	45	20	19	7	2	0	-1
14	Dry whey	5 hours	16	43	6	10	4	-3	-2	1
15	Dry whey	2 hours	60	45	13	14	-1 <sup>4</sup>			
16	Dry whey	2 hours <sup>5</sup>	8	45	10	4	-9	-9		

<sup>1</sup> Yeast 2—pure dry yeast.<sup>2</sup> Yeast 3—dehydrated yeast.<sup>3</sup> Yeast 4—fresh baker's yeast.<sup>4</sup> Animals used for other tests as soon as weight remained constant or decreased.<sup>5</sup> Sucrose replaces dextrin.

receiving the autoclaved skim milks were quite similar to those obtained from dry whey. When dextrin is used as the carbohydrate in the basal ration, the results are about the same as those obtained when sucrose is used, although the average length of life on the vitamin B deficient dextrin ration is a few days longer, and somewhat more variable.

The explanation of the observed difference in the growth reaction of the animals receiving autoclaved yeast and those receiving the autoclaved milk products must remain a matter of speculation. The results from the autoclaved yeast are in

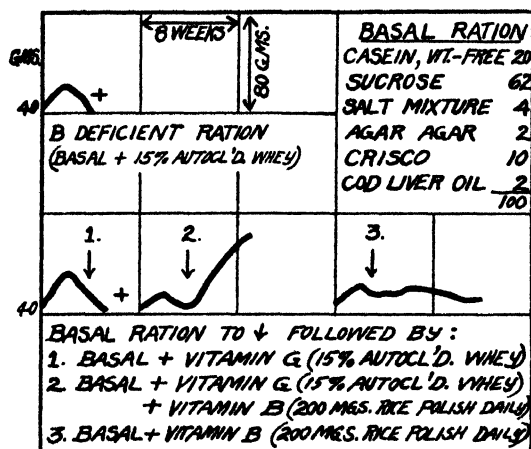


Chart 1 Growth response of rats receiving the basal ration with and without vitamin B and G supplements.

harmony with those reported by Sure ('33). However, other investigators, such as Chase and Sherman ('31), Evans and Lepkovsky ('31) and Kemmerer and Steenbock ('33) report results and growth reactions from autoclaved yeast similar to those reported herein from the autoclaved milk products. The consistency and satisfaction of the results from 15 per cent dry whey autoclaved for 2 hours as a source of vitamin G free from vitamin B have been such as to warrant its extended use for vitamin B assays made at this laboratory. The following is an outline of the procedure which has been successfully used for some time past.

Dry whey<sup>3</sup> is moistened with water to a paste of medium consistency and autoclaved for 2 hours at 120°C. in shallow containers, the paste being about 2 inches in depth. As soon as it is taken from the autoclave it may be intimately mixed with dextrin, and air or oven dried; or it may be spread in thin layers and dried at 60° to 100°C. and subsequently ground. The dry material is incorporated in the basal ration previously described to the extent of 15 per cent, replacing an equivalent amount of dextrin or sucrose. Some samples of the particular dry whey under consideration have been quite free from vitamin B when received, while other samples have been found to contain variable amounts of this factor. Autoclaving should, therefore, be resorted to as a precautionary measure. Growth of the animals on the basal ration containing the autoclaved dry whey usually continues for about 21 days, if a casein entirely free from vitamin B is used. At the end of the vitamin B depletion period, as indicated by decline in weight, the substance to be assayed for vitamin B is fed as a daily supplement while continuing the B deficient ration.

#### SUMMARY

1. Dry whey autoclaved for 2 hours at 120°C. contains no vitamin B as indicated by the growth results from white rats.

2. Fifteen per cent of the autoclaved dry whey used in these experiments supplies an amount of vitamin G sufficient for good growth.

3. More consistent and satisfactory results have been obtained at this laboratory from autoclaved dry whey, as a source of vitamin G free from vitamin B, than from autoclaved yeast.

<sup>3</sup> The dry whey used for these experiments is known commercially as Peeble's Lacto-Milk and is distributed by the Western Condensing Co., San Francisco, California.

## LITERATURE CITED

- BENDER, R. C., AND G. C. SUPPLEE 1932 The vitamin B and G content of dry skimmed milk and dry whey. *J. Dairy Sci.*, vol. 15, p. 445.
- BOOHER, L. E. 1933 The concentration and probable chemical nature of vitamin G. *J. Biol. Chem.*, vol. 102, p. 39.
- CHASE, E. F., AND H. C. SHERMAN 1931 A quantitative study of the determination of the antineuritic vitamin B. *J. Am. Chem. Soc.*, vol. 53, p. 3506.
- CHICK, H., AND M. H. ROSCOE 1929 A method for the assay of the antineuritic vitamin B, in which the growth of young rats is used as a criterion. *Biochem. J.*, vol. 23, p. 498.
- EVANS, H. M., AND S. LEFKOVSKY 1931 On the preparation of a concentrated source of the heat-labile vitamin B, free from contamination with the heat-stable factor G. *J. Nutrition*, vol. 3, p. 353.
- GUERRANT, N. B., AND R. A. DUTCHER 1932 The assay of vitamins B and G as influenced by coprophagy. *J. Biol. Chem.*, vol. 98, p. 225.
- KEMMERER, A. R., AND H. STEENBOCK 1933 A study of the sparing action of fats on the vitamin B content of animal tissues. *J. Biol. Chem.*, vol. 103, p. 353.
- KUHN, R., H. RUDY AND TH. WAGNER-JAUREGG 1933 Über lacto-flavin (vitamin B<sub>2</sub>). *Ber. Chem. Ges.*, Bd. 66, S. 1950.
- ROSCOE, M. E. 1930 The distribution of the vitamin B complex. I. Leafy vegetables. *Biochem. J.*, vol. 24, p. 1754.
- SUPPLEE, G. C., O. J. KAHLBERG AND G. E. FLANIGAN 1931 The growth promoting properties (vitamin B complex) of the concentrated water soluble portion of milk. *J. Biol. Chem.*, vol. 93, p. 705.
- SURE, B. 1933 A highly satisfactory vitamin B deficient ration. *Proc. Exp. Biol. and Med.*, vol. 30, p. 779.





# THE VITAMIN B SUPPLEMENTATION OF MILK

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## ONE CHART

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The primary results, and in many instances the deductions from published data, clearly show that vitamin B directly affects growth and efficiency of food utilization (Graham and Griffith, '33; Sure, '32; Sherman, '31). An analysis of certain data (Coward et al., '33; Graham and Griffith, '33) seems to support the view that there is a variable complementing effect between dietary constituents, vitamin in character or otherwise, and vitamin B which may account for variations in growth rate. Whether the observed growth is always due to the particular vitamin specified, or whether it is due to particular balanced relationships between a plurality of vitamins may be a matter of conjecture in many instances. Notwithstanding the lack of suitable methods necessary to determine the specific mode of action of vitamins B and G, an accelerated rate of growth of white rats has been obtained at this laboratory for many years from milk and its derivatives supplemented by substances known to contain relatively large amounts of vitamin B. The data reported herein are concerned with variations in the growth rate as affected by vitamin B with and without accompanying milk solids.

## EXPERIMENTAL

It has been shown previously (Supplee et al., '31) that crude rice polish and a water soluble vitamin concentrate prepared

from milk, when supplementing a suitable basal ration, permitted excellent growth; in one instance a gain of 59 gm. per week was noted. Seven per cent of rice polish and an equal amount of the milk vitamin concentrate have supported, up to the present time, normal growth, reproduction and lactation in white rats through eight successive generations. Such animals as it has been practicable to retain for the purpose of determining longevity on this diet have shown an average age at the time of death of 114 (maximum 152) weeks for the females and 88 (maximum 133) weeks for the males. While this ration has not been assayed to determine its relative vitamin B and G content in terms of empirical units, it is known to contain relatively high proportions of each.

In view of these and other data which have been obtained from milk and rice polish or their derivatives, the test substances required for the following studies were confined to these two products. The plan of procedure was to determine the relative vitamin B content of rice polish derivatives and milk solids according to the Chase and Sherman ('31) assay procedure. The assayed substances were then fed in varying amounts, singly and in combination, for the purpose of ascertaining the growth response from different amounts or 'units' of the vitamin; the same technic as used for the assays was followed.

The basal ration free from vitamin B and G consisted of vitamin-free casein,<sup>1</sup> 20 parts; powdered agar agar, 2 parts; Steenbock ('23) salt mixture, 4 parts; dextrin, 63 parts; Crisco, 10 parts; and cod liver oil, 2 parts. This ration was converted to a vitamin B free ration containing an adequacy of vitamin G by replacing 15 parts of dextrin with an equal

<sup>1</sup> The casein used in this ration is obtained free from the water soluble vitamins during direct preparation from fluid milk by controlling the ionic condition of the aqueous suspension. It is not subjected at any time during the course of preparation to the solvents usually used in the treatment of commercial grades of casein for the purpose of rendering them vitamin-free. This product has been commercially available for about 2 years and is distributed by the Casein Manufacturing Company of America, New York.

amount of autoclaved dry whey.<sup>2</sup> The depletion period on this ration is about 3 weeks, at which time weight of the rats is from 60 to 80 gm. If it is desired to convert the basal ration to one free, or substantially free, from vitamin G, but with sufficient vitamin B for normal growth, 2 per cent crude rice polish is added.

Aqueous extracts were prepared from crude rice polish by violently agitating 1 part of the polish with 4 parts of water at 7°C. for 4 hours. The insoluble matter was removed by mechanical means leaving a solution containing from 4 to 5 per cent dry matter. Assays of this material showed that about 25 mg. of dry substance per day were required to promote a 3 gm. gain per week for a period of 6 weeks.<sup>3</sup> A more highly purified concentrate<sup>4</sup> prepared from rice polish according to the method described by Sure ('32) required 2.8 mg. of dry substance for the same growth response. Assays of the dry milk solids<sup>5</sup> showed that 270 mg. were required for the 3 gm. gain in weight.

In order to eliminate certain influences which might effect the interpretation of the results obtained from feeding mixtures of the vitamin B concentrates and milk solids, it was desirable to determine whether the freshly prepared aqueous extract underwent deterioration in potency during the heat treatment to which milk is subjected in the atmospheric double roller process of desiccation. Supersaturated solutions of pure lactose varying from 27 to 40 per cent concentration were made with the freshly prepared water extract of

<sup>2</sup> It has been found that one particular brand of dry whey frequently contains little or no vitamin B. Complete absence of this vitamin in different shipments cannot be relied upon. Therefore it is our practice to autoclave the moistened product for 2 hours at 120°C. This product is known as Peeble's Lacto Milk and is distributed by the Western Condensing Co., San Francisco, California.

<sup>3</sup> The crude rice polish from which these extracts were prepared required 100 mg. for 1 unit. Other samples of crude rice polish have been more potent in vitamin B, requiring only 60 to 75 mg. per unit.

<sup>4</sup> We are indebted to Dr. L. W. Bass, of the Borden Research Department, for furnishing a sample of this concentrate prepared by Doctor Sure.

<sup>5</sup> The dry milk used throughout the experiments recorded in this paper is known as Dryco Brand. It is irradiated by ultraviolet rays prior to desiccation by the Just Process.

rice polish. These solutions were converted to dryness by proper manipulation of the drying apparatus and yielded the beta type lactose impregnated with known amounts of the rice polish extract solids. The temperature to which the mixture was subjected varied progressively from about 110°C. to 135°C. during a period of about 9 seconds. The vitamin B assays of various samples of the heat treated product showed that substantially 28 mg. of the extract solids were required for 1 rat unit, as compared with 25 mg. required prior to heating. The daily amount of this vitamin B fortified lactose necessary to carry the extract solids for 1 unit varied from 300 to 500 mg., depending upon the initial concentration. On the basis of these results as well as other data, it is concluded that the destruction of vitamin B during the heat treatment was relatively slight or at least not greater than 10 per cent.

The assay results from the different test substances were used as a guide for determining an appropriate range of graduated doses of vitamin B which it was desired to feed singly or in combination. Since it was desired to maintain the amount of growth during the test period within a range which would not be in the least affected by maturity size, fractions of the amount required for the 3 gm. unit gain or small multiples thereof were used. The test substances were supplied as daily supplements following the depletion period. All experimental conditions were maintained the same as prevailed during the determination of the assay results. Where dry milk solids were used singly or in combination with rice polish extracts, the test substances were subjected to the heat treatment prevailing in the desiccation of milk by the roller process. Where rice polish concentrates were fed alone, desiccation of the fluid extracts was accomplished by drying with the basal ration at a temperature not exceeding 80°C. or by concentrating in vacuum and subsequently drying by the spray method commonly employed in the drying of milk. The only exception to this procedure was in the case where the aqueous extracts were dried with the lactose solutions in the manner

mentioned above. Preliminary tests showed no significant difference in potency resulting from these methods of desiccation. The averages of the results obtained are recorded in table 1 and significant relationships are shown graphically in chart 1. In this chart the expected normal rate of growth is plotted (dotted line) on the assumption that each rat unit

TABLE 1  
*Growth response due to vitamin B*

MATERIAL FED AS DAILY SUPPLEMENT	SUPPLEMENTING MATERIAL FED PER DAY			NUMBER OF RAT UNITS OF VITAMIN B <sup>1</sup>	AVERAGE GAIN PER WEEK	FOOD INTAKE PER GRAM GAIN
	Total solids	Milk solids	Rice polish extract solids			
	mg.	mg.	mg.		gm.	gm.
Water extract of rice polish	9.4		9.4	0.38	0.4	46.3
Water extract of rice polish	11.7		11.7	0.47	0.6	17.8
Water extract of rice polish	13.9		13.9	0.56	2.4	9.0
Water extract of rice polish	18.8		18.8	0.75	1.6	9.8
Water extract of rice polish	23.4		23.4	0.94	4.2	5.9
Water extract of rice polish	27.7		27.7	1.11	5.3	4.7
Water extract of rice polish	37.7		37.7	1.51	6.3	3.3
Dry milk solids	200.0	200		0.70	2.5	11.0
Dry milk solids	400.0	400		1.50	3.9	7.9
Dry milk solids	800.0	800		3.00	9.9	4.2
Water extract of rice polish + dry milk	100.0	95	5.0	0.55	1.1	20.8
Water extract of rice polish + dry milk	200.0	190	10.0	1.10	4.5	6.5
Water extract of rice polish + dry milk	400.0	380	20.0	2.20	8.8	4.1
Vitamin B concentrate (Sure)	0.5		0.5	0.18	Died	....
Vitamin B concentrate (Sure)	1.0		1.0	0.36	1.2	18.3
Vitamin B concentrate (Sure)	2.0		2.0	0.72	1.1	23.2
Vitamin B concentrate (Sure)	3.0		3.0	1.07	4.3	6.2
Vitamin B concentrate (Sure) + dry milk	200.5	200	0.5	0.91	3.6	7.2
Vitamin B concentrate (Sure) + dry milk	201.0	200	1.0	1.08	6.5	4.7
Vitamin B concentrate (Sure) + dry milk	202.0	200	2.0	1.42	8.7	3.8
Vitamin B fortified lactose	279.0		20.0	0.71	2.5	9.9
Vitamin B fortified lactose	360.0		26.0	0.93	3.8	6.4
Vitamin B fortified lactose	558.0		40.0	1.39	7.5	4.4
Vitamin B fortified lactose	720.0		52.0	1.86	8.6	3.9

<sup>1</sup> Calculated on basis of amount necessary for 1 rat unit: 25 mg. rice polish extract solids. 28 mg. rice polish extract solids (fortified lactose). 2.8 mg. Sure's vitamin B concentrate. 270 mg. milk solids.

produces a 3 gm. gain per week. It is a reference line which is included for comparative purposes only. The slope of the other lines was calculated from the various data according to the general formula ( $y = mx - n$ ).

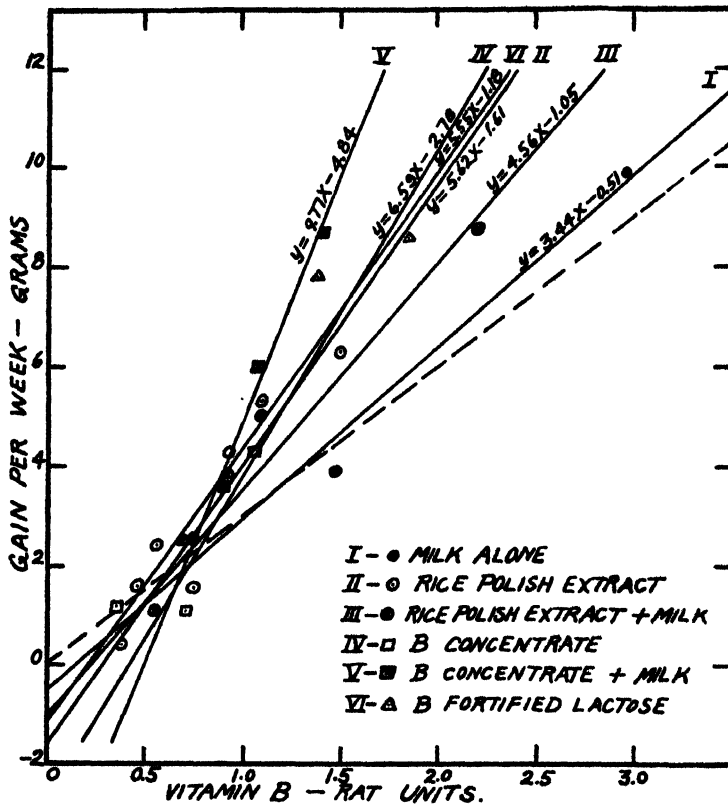


Chart 1 Relation of vitamin B to growth response of various rice polish substances and milk, alone and combined.

The tabular data, and especially the graphs show a proportional relationship between the rate of growth and the vitamin B intake. In all instances, however, with the possible exception of the results obtained from milk alone, the growth rate is greater than that which was to be expected by calculation. The results from the aqueous extracts of rice polish (curves II and VI) and the rice polish concentrate

prepared according to the method of Sure (curve IV) show growth promoting properties of the same order of magnitude when the 3 gm. unit gain is the basis of appraisal of their vitamin B content. However, it cannot be assumed that the similarity of results from these three substances is sufficient to warrant the firm conclusion that the observed rate of growth represents a specific and inflexible criterion which may be expected from like amounts of vitamin B accompanying substances of heterogeneous composition.

The different rates of growth obtained from these vitamin B concentrates when fed with milk are interpreted as evidence of a variable growth promoting effect resulting from different proportions of vitamin B and other constituents of the diet, vitamin in character or otherwise. This observation seems to be supported by the recent work of Coward ('33) and is in conformity with previous results obtained at this laboratory (Supplee, '31). The data show that the lowest growth rate per unit of vitamin B was obtained from the unsupplemented milk (curve I); the highest rate was from the milk supplemented by the highly purified Sure concentrate (curve V); an intermediate rate of growth was obtained from the milk supplemented by the less potent aqueous extract of rice polish. While the growth rate resulting from this last test mixture was substantially greater than from milk alone, it was lower than that obtained without the accompanying milk solids.

While a substantial degree of tolerance must be allowed for the mathematical presentation of biological data of this character, the results seem to leave no reasonable doubt that definite differences in growth promoting properties are exhibited by the supplemented and unsupplemented products. Since the unit amount of vitamin B was determined by unit gain in weight, it is obvious that if different substances or variations in the optimal balance between substances affect the rate of growth, a false criterion for vitamin B will result. For example, the rate of growth shown by the unsupplemented aqueous extract of rice polish may have been due to not only



its vitamin B content but to other accompanying substances as well. However, the experimental plan and method of expressing the data called for uniformity in assuming that growth response resulting from the assay procedure was entirely attributable to vitamin B. When this product supplemented milk, only its actual vitamin B content would be manifested; this was apparently lower than was seemingly revealed when it was fed alone. The results from the more highly purified Sure concentrate which presumably contained less growth promoting substances other than vitamin B, seem to be in harmony with this explanation.

The data as a whole show that rate of growth is increased and greater efficiency of food utilization results from increasing amounts of vitamin B provided in the ration. These manifestations bear a direct linear relationship to the amount of vitamin B present, irrespective of supplementing milk solids. The results from extended ad libitum feeding experiments with dry milk supplemented with crude rice polish or its aqueous extracts support the data reported herein, in showing substantially the same linear relationship between the amount of vitamin B supplied and the growth rate. When 10 per cent unsupplemented dry milk solids were used in the ration the average gain was 8.9 gm. per week; when a further supplementation of 2, 3 or 4 per cent of rice polish or its equivalent aqueous extract was provided, the average gain per week was 13.6, 15.5 and 18.7 gm., respectively. While the various data show a direct relationship between growth rate and the amount of vitamin B supplied, a specified rate of growth cannot be accepted as an infallible criterion for judging the absolute amount of vitamin B present.

Although milk is not generally considered to be a rich source of vitamin B from a comparative standpoint, human experience has shown no general evidence of an acute deficiency of this factor in the normal milk fed infant. The data presented herein would seem to show that the amount of vitamin B required to maintain a given rate of growth may be variable depending upon the presence of other dietary sub-

stances. The results indicate that substances which have a sparing effect upon the vitamin B demand are contained in milk, or else, the particular balanced relationship between the vitamin B and other factors contained in milk are such that the nutritive character of milk as a whole cannot be considered as particularly deficient in this vitamin. Nevertheless, various observers (Hoobler, '28; Dennett, '29; and Barry, '30) have noted that the plane of nutrition of infants and children may be improved, in so far as gain in weight may be accepted as a valid criterion, by supplementing the milk dietary with vitamin B. An extensive clinical study of the merits of vitamin B fortified dry milk similar to that used in these experiments has been recently reported by Gaynor and Dennett ('34), wherein it is stated that: "This increase in weight (referring to the gain made by infants receiving the vitamin B fortified milk) is not due to an increased caloric intake as demonstrated by the fact that the average daily intake per pound body weight in the vitamin B group is 35 as compared with from 45 to 55 in other groups."<sup>6</sup> This observation is of particular interest in view of the data recorded in this paper wherein the growth promoting properties of the same product were subjected to an integrated study with experimental animals.

#### CONCLUSIONS

1. Potent vitamin B extracts may be prepared from rice polish by simple extraction procedures; such concentrates may be condensed in vacuum, spray dried, or dried by the Just Process without significant diminution in potency.

2. There is a direct linear relationship between the rate of growth of white rats and the amount of vitamin B supplied in the ration. However, a specified rate of growth cannot be accepted as an infallible criterion for judging the absolute amount of vitamin B present; the presence of other dietary constituents or definite relationships between such constitu-

<sup>6</sup> We are indebted to Doctors Gaynor and Dennett for the privilege of reading their manuscript now in press.

ents and the amount of vitamin B seem to determine the rate of growth observed.

3. Dry milk containing a concentrated water extract of vitamin B obtained from rice polish showed greater growth promoting properties than was shown by the milk not containing such supplement.

4. Vitamin B, either with or without milk supplementation, increases the efficiency of food utilization as judged by the growth response per unit of food intake.

5. A highly purified vitamin B concentrate prepared from rice polish gave results comparable to those obtained by simple water extraction; however, the former was approximately ten times more potent than the latter as judged by its effectiveness when used with milk or when assayed without the milk supplement.

The authors acknowledge the technical assistance rendered by Dr. Stefan Ansbacher and Mr. M. R. Simonds, in carrying out the work reported in this paper. Suggestions concerning the presentation of these data by Dr. G. R. Cowgill and Dr. L. B. Mendel are also gratefully appreciated.

## LITERATURE CITED

- CHASE, E. F., AND H. C. SHERMAN 1931 A quantitative study of the determination of the antineuritic vitamin B. *J. Am. Chem. Soc.*, vol. 53, p. 3506.
- COWARD, K. H., J. H. BURN, H. W. LING AND B. G. E. MORGAN 1933 The determination of the antineuritic vitamin. *Biochem. J.*, vol. 27, p. 1719.
- DENNETT, R. H. 1929 Routine use of the vitamin B factor in infant feeding. *J. Am. Med. Assoc.*, vol. 92, p. 769.
- GAYNOR, M. F., AND R. H. DENNETT 1934 Rice polish as a source of vitamin 'B' complex. *J. Pediatrics*, vol. 4, p. 507.
- GRAHAM, C. E., AND W. H. GRIFFITH 1933 Studies on growth. II. The effect of vitamins B and G on the consumption and utilization of food. *J. Nutrition*, vol. 6, p. 195.
- HOOBLER, B. R. 1928 Symptomatology of vitamin B deficiency in infants. *J. Am. Med. Assoc.*, vol. 91, p. 307.
- MORGAN, A. F., AND M. M. BARRY 1930 Underweight children: Increased growth secured through the use of wheat germ. *Am. J. Dis. Child.*, vol. 39, p. 935.
- SHERMAN, H. C., AND M. R. SANDELS 1931 Further experimental differentiation of vitamins B and G. *J. Nutrition*, vol. 3, p. 395.
- STEENBOCK, H., AND E. M. NELSON 1923 Fat-soluble vitamin. XIII. Light in its relation to ophthalmia and growth. *J. Biol. Chem.*, vol. 56, p. 362.
- SUPPLEE, G. C., O. J. KAHLENBERG AND G. E. FLANIGAN 1931 The growth promoting properties (vitamin B complex) of the concentrated water soluble portion of milk. *J. Biol. Chem.*, vol. 93, p. 705.
- SURE, B. 1932 Avitaminosis. XI. The specific effect of vitamin B on growth as evidenced by the use of vitamin B concentrates. *J. Biol. Chem.*, vol. 97, p. 133.
- SURE, B., M. C. KIK AND M. E. SMITH 1932 The specific effect of vitamin B on growth. *J. Nutrition*, vol. 5, p. 155.



# THE NUTRITIVE VALUE OF LACTOSE

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ONE FIGURE

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The fact that lactose passes at least in part unabsorbed to the large bowel, raises the question of its nutritive value. Textbooks frequently state that lactase is present during infancy, but disappears, particularly in man, during later life. Evidence on this latter point, however, to our knowledge is very meager. That lactose passes through to the large bowel is demonstrated by its action on the bacterial flora (Rettger and Cheplin, '21; Mitchell, '27), lactic acid production and its effect on the pH of the colon contents (Robinson and Duncan, '31; Kline et al., '32), and the apparently secondary effect on calcium and phosphorus assimilation (Kline et al., '32; Bergeim, '26; Robinson et al., '29). It seems logical to assume that the nutritive value of lactose is inversely proportioned to its bacterial utilization and fermentation in the colon unless some of the intermediary products of fermentation, such as lactic acid, are reabsorbed from the colon and used as fuel in the body.

The fact that lactose cannot be used as such by the animal organism was first shown by Voit (1897) in demonstrating that lactose administered subcutaneously was practically completely eliminated in the urine. The fact that lactose was also poorly used when orally administered as far as glycogen formation in the dog was concerned was first shown by Murschhauser ('11). He found that 50 gm. of sugar in 8

hours produced only a value of 6 for lactose as compared to 100 for glucose. These findings were consistent with those of Lusk ('15) that lactose in the dog failed to produce the specific dynamic effect shown by sucrose or glucose and failed to materially increase the respiratory quotient. Lusk concluded that lactose was not oxidized at all and that this was due to the absence of lactase in the intestine. In the phlorhizinized dog, however, Deuel and Chambers ('25) found that glucose could be formed, at least in part, from lactose. In their experiments there was a delay in the elimination of the 'extra sugar' after lactose ingestion as compared with glucose, fructose and galactose. Only 50 per cent of the ingested lactose was excreted as glucose, while that for the other sugars was practically 100 per cent.

The rat, on the other hand, appears to be more capable of utilizing lactose. Greisheimer and Johnson ('30) showed that, after feeding rats for 16 to 18 days on diets containing 87.5 per cent of the total caloric value in sugar, the liver glycogen (3.15 per cent) on the lactose diet was only 62 per cent of the liver glycogen (4.89 per cent) on glucose or sucrose diets. Mitchell ('27) studied the growth of the rat on diets containing from 30 to 60 per cent carbohydrate and found growth on 30 per cent lactose was only 50 per cent of that on the other carbohydrates. She noted that the per cent of food sugar lost in the feces on diets as high as 60 per cent carbohydrate was only 3.12 per cent greater for lactose than for sucrose. These experiments, however, did not answer the question of the nutritive value of lactose, inasmuch as the total amount of food eaten was not reported. Furthermore, the growing animal does not lend itself to this type of experiment, inasmuch as it has been shown that lactose may affect the rate of skeletal growth (Kline et al., '32).

#### PLAN OF EXPERIMENTS

Young full-grown rats were fattened on a calf meal diet<sup>1</sup> and then put in small individual cages and placed on daily

<sup>1</sup> 'G. L. F. calf meal' P-18, F-4, C-56, indigestible 22.

weighed diets approximately 30 to 40 per cent below maintenance for each rat. They were weighed every third day and in the course of 20 to 40 days lost from 40 to 50 gm. body weight. After this weight loss, lactose, glucose or sucrose was added to the diet in an amount represented by the difference between the maintenance and sub-maintenance reducing diet. Thus, if 100 parts of meal represented the maintenance diet and 65 the reducing diet, the amount of sugar added was 35. Each rat was given a daily weighed portion of the meal-sugar mixture equivalent to 100 parts instead of 65 of the meal alone on the reducing diet. In this manner, the protein, fat and other dietary constituents were unchanged during the reducing and weight-gaining periods, the only difference being the sugar added during the second period of the experiments.

Since the caloric intake of the rats was constant during the periods of lactose, glucose and sucrose feeding, any difference in weight curves should be due to variation in the extent of assimilation of the sugars unless there was a concomitant change in total activity of the animals. To control this latter factor a separate set of experiments was carried out to study the voluntary activity of the rats on the glucose, lactose and sucrose diets. A group of ten rats was placed individually in the conventional revolving cages of a circumference of 1 meter. They were given daily 15 gm. of a diet consisting of 65 parts meal and 35 parts sugar. The food not consumed was weighed and the daily food consumption calculated.

#### RESULTS

The results of the weight gaining experiments are graphically shown in figure 1. Curve 1 shows the average weights of nine rats during the latter third of the weight losing period and during the period when glucose was added. Curve 2 shows the comparable results in the averages of ten rats when the equivalent amount of lactose was substituted. After 32 days on the glucose diet the gain in weight was 35 gm., while on the lactose diet the gain was only 22.5 gm. the equivalent of only 64 per cent of the former.



In curves 3 and 4 are shown the comparable average weight gains of groups of rats of ten each on sucrose and lactose diets. In general, the glucose and sucrose curves are approximately alike. In 21 days the weight gain of the rats on the sucrose diet was 33 gm., while on the lactose diet the gain was only 20 gm.—representing only 61 per cent of the sucrose gain. In the group of rats on the lactose diet represented by

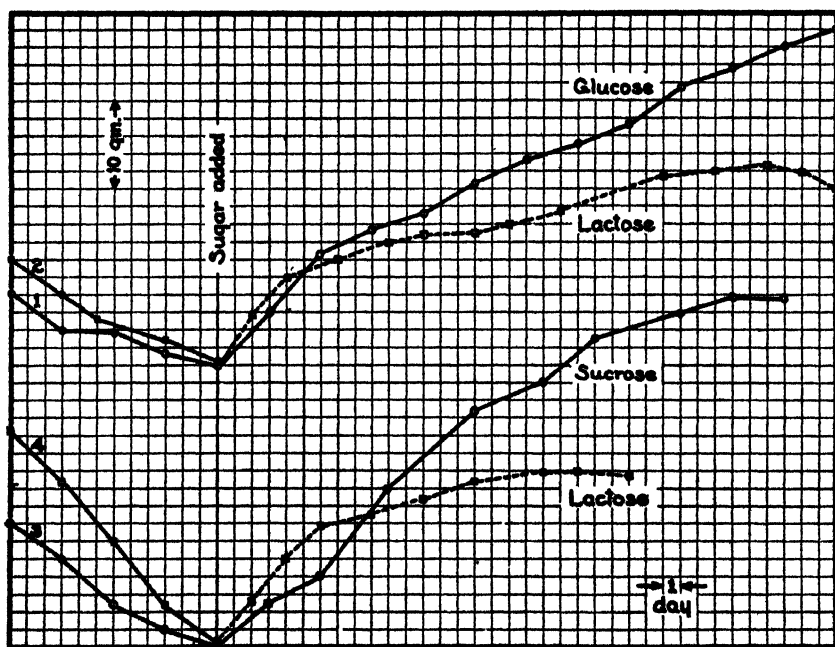


Figure 1

curve 4, the experiment was terminated at the end of 24 days, because of the refusal of the rats to eat all the diet. At no time was a diarrhea noted while on the high carbohydrate diets. Only traces of fermentable reducing substances were found in the feces after acid hydrolysis and protein removal during the periods of lactose feeding. On her 60 per cent lactose diets, which was a somewhat higher percentage of sugar than we used, Mitchell found a daily excretion per rat of 172 mg. per cent reducing substances expressed as sugar.

Since the acid hydrolysis of feces produces non-fermentable reducing substances, the values found by Mitchell and expressed as sugar are probably too high.

That the relative decrease in the weight gain on lactose as compared with glucose or sucrose was not due to increased voluntary activity is shown in table 1. Young rats nearly full grown were used, inasmuch as it is difficult to train full grown rats to run in the exercise cages. Meal was fed during the

TABLE 1  
*The effect of lactose feeding on the voluntary activity of nine rats*

DATE, 1933	WEIGHT (AVERAGE), GRAMS	FOOD CONSUMED PER DAY (AVERAGE), GRAMS	REVOLUTIONS PER DAY (AVERAGE)
Meal only			
7-3 to 7-18			100
Meal 65, glucose 35			
7-18 to 7-21	206	18.3	95
7-21 to 7-25	221	15.7	120
7-25 to 7-30	221	13.9	120
7-30 to 8-5	220	13.9	128
8-5 to 8-11	224	15.1	122
8-11 to 8-17	220	14.0	125
Average	219	15.1	118
Meal 65, lactose 35			
8-17 to 8-18	218	12.6	122
8-18 to 8-25	223	13.3	105
8-25 to 9-1	209	13.2	95
9-1 to 9-8	208	13.2	97
Average	214	13.1	105

first period of 2 weeks until the rats reached a level of uniform activity. Following this the rats were placed on a 26-day period of glucose meal which was succeeded by a 20-day period of lactose meal.

During the lactose period there was at first a gain and then a progressive drop in weight in the averages of the nine rats. This initial relative gain and subsequent loss in weight on lactose as compared with glucose and sucrose is also indicated in the groups shown in figure 1. Both average food

intake and voluntary activity decreased on the lactose period as compared with the glucose period. These experiments showed quite conclusively that the relative weight loss or diminished weight gain on the lactose diets was not due to increased voluntary activity.

The possibility that hydration changes may have entered into the differences between lactose and glucose or sucrose weight gain has not been ruled out in these experiments. Since the respective differences in weight were 36 and 39 per cent, it is improbable that there was this degree of dehydration on the lactose diets.

#### SUMMARY

The nutritive value of lactose in the rat as compared with that of glucose and sucrose was studied by noting the weight gain after the sugars were added in equivalent amounts to a sub-maintenance diet.

The weight gain after lactose was added to the diet was at first slightly greater than that after glucose or sucrose addition, but after the fifth to the ninth day the weight gain on the lactose diet was distinctly less than that for the other two sugars. After 32 days, it was only 64 per cent of the gain on glucose and after 21 days, only 61 per cent of the gain on the sucrose diet.

On the lactose diets the weight gain curves had a tendency to flatten out considerably before those for glucose or sucrose. The level of the flattening of the lactose curve was 50 per cent below that of the sucrose curve.

The voluntary activity of rats on a lactose diet was not greater than that of a group on a glucose diet. Activity, therefore, is not a factor in the poorer nutritive value of lactose.

A considerable portion of ingested lactose, approximately from 40 to 50 per cent, may be lost to the rat as far as weight or energy relationships are concerned.

## LITERATURE CITED

- BERGEIM, O. 1926 Calcium absorption. *Proc. Soc. Exp. Biol. Med.*, vol. 23, p. 727.
- DEUEL, H. J., AND W. H. CHAMBERS 1925 The rate of elimination of ingested sugars in phlorhizin diabetes. *J. Biol. Chem.*, vol. 65, p. 7.
- GREISHEIMER, E. M., AND O. H. JOHNSON 1930 Glycogen formation in rats. III. Diets containing 87.5 per cent of total caloric value in lactose, glucose and sucrose. *Proc. Soc. Exp. Biol. Med.*, vol. 27, p. 769.
- KLINE, O. L., J. A. KEENAN, C. A. ELVEHJEM AND E. B. HART 1932 Lactose in nutrition. *J. Biol. Chem.*, vol. 98, p. 121.
- LUSK, G. 1915 Animal calorimetry. An investigation into the causes of the specific dynamic action of the foodstuffs. *J. Biol. Chem.*, vol. 20, p. 555.
- MITCHELL, H. S. 1927 Comparative physiological values of different amounts of lactose, based on growth and fecal analysis. *Am. J. Physiol.*, vol. 79, p. 542.
- MURSCHAUSER, H. 1911 Über die Ausnutzung der verschiedenen Zuckerarten zur Glycogenbildung in der Leber. *Pflügers Arch. der Physiol.*, Bd. 139, S. 255.
- RETTGER, L. F., AND H. A. CHEPLIN 1921 A treatise on the transformation of the intestinal flora. Yale Univ. Press, New Haven.
- ROBINSON, C. S., AND C. W. DUNCAN 1931 The effect of lactose and the acid-base value of the diet on the hydrogen ion concentration of the intestinal contents of the rat and their possible influence on calcium absorption. *J. Biol. Chem.*, vol. 92, p. 435.
- ROBINSON, C. S., C. F. HUFFMAN AND M. F. MASON 1929 The results of the ingestion of certain calcium salts and of lactose. *J. Biol. Chem.*, vol. 84, p. 257.
- VOIT, F. 1897 *Deut. Arch. klin. Med.*, Bd. 58, S. 523. Quoted from Lusk, *The Science of Nutrition*, p. 319, 4th ed. The W. B. Saunders Co., Philadelphia.



# IRRADIATED VITAMIN B COMPLEX AND DERMATITIS

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## ONE FIGURE

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Hogan and Hunter ('28) observed that one member of the vitamin B complex is destroyed by ultraviolet irradiation. The original procedure was modified by Hogan and Richardson ('32) and they observed that rats which receive irradiated dried yeast develop a severe dermatitis. In our experience the incidence of dermatitis and the percentage of mortalities have both been practically 100 per cent. After this degree of success had been attained, it seemed desirable to reexamine the technic in an effort to effect additional improvements and to eliminate any precautions that might prove unnecessary. The purpose of this paper is to describe the technic now in use, though the earlier publications should be consulted for complete details.

## EXPERIMENTAL

Albino rats are used as experimental animals. The litters we expect to use, with their mothers, are transferred to floors of metal screens, without bedding, usually at 15 days of age. If a litter is under the normal weight this transfer may be delayed 1 or 2 days. From this time on the mother and her young are supplied with the experimental diet, which is free

<sup>1</sup>Contribution from the Departments of Animal Husbandry and Agricultural Chemistry, Missouri Agricultural Experiment Station. Journal series no. 376.

of the antidermatitic factor. The litters are usually weaned at 21 days but if they weigh less than 25 gm. at that time they are left with their mothers until they do attain that weight. Immediately on weaning, the young are transferred to individual cages and the experimental period begins.

The experimental ration formerly used, no. 1545, is made up as follows: Casein, 20; sucrose, 71; cellulose, 3; salt mixture (Osborne and Mendel, '19), 4; cod liver oil, 2. The casein had been thoroughly extracted with acidified water, with alcohol and with ether, and the sucrose had been recrystallized. The food is supplied *ad libitum*, and no records are taken as to the amount consumed.

The results thus far have been most consistent when dried yeast<sup>2</sup> was the source of vitamin B. During irradiation this is spread out in a thin layer, and stirred thoroughly at least four times. The distance from the arc to the yeast is 14 cm., and irradiation is continued for 10 hours. The yeast supplement for each animal is weighed out in a small glass container and usually it is consumed immediately. Except in special cases, the daily allowance is limited to 100 mg. When receiving this material rats quickly develop severe dermatitis, and the survival periods are short. A photograph of a typical case of dermatitis is reproduced in figure 1.

#### STUDIES ON THE PROCEDURE

1. *Intensity of irradiation.* This portion of the technic has not been changed. If the period of irradiation is too short, there will not be sufficient destruction of the antidermatitic factor; and if too prolonged, it is possible that there may be too much destruction of the antineuritic factor.

2. *Preliminary preparation.* Originally the experimental animals, with their mother, were placed on screens at 5 days of age. At 15 days the mother and litter were given the experimental ration, and the litter was weaned at 21 days. Somewhat later, the transfer of the litter to screens was delayed until the fifteenth day, with no appreciable effect on

<sup>2</sup> Secured from the Harris Laboratories, Tuckahoe, New York.

the results. A study was then made to see if the preliminary preparation could be dispensed with entirely, but it was decided this was not advisable. A delay of 6 days in subjecting the animals to experimental conditions postpones the appearance of dermatitis, and lengthens the survival period. Our observations on this point are summarized in table 1. The vitamin carrier was dried yeast.

3. *Purification of the protein.* Casein which had been leached with acid water only was substituted for the prepa-

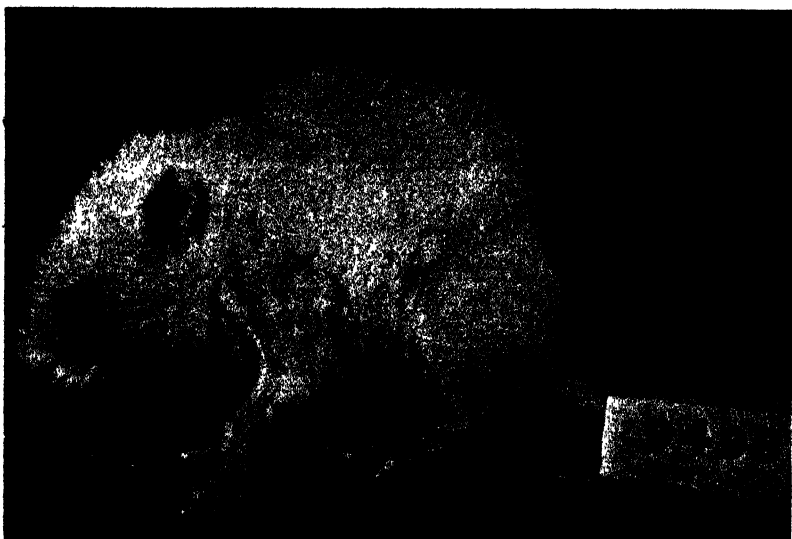


Fig. 1 Characteristic lesions have appeared on the feet, nose, and mouth, but there is no loss of fur elsewhere. The eyelids are adherent.

ration which had also been extracted with alcohol and ether, but more consistent results were obtained when the alcohol and ether extractions had not been omitted.

4. *Influence of carbohydrate component.* Commercial sucrose has been substituted for the recrystallized preparation. At first there was reason to believe this substitution may have lengthened slightly the survival periods. We are now using commercial sucrose exclusively, and find that for our purpose it is only slightly, or not at all, inferior to the recrystallized preparation. An attempt was also made to use



cornstarch<sup>3</sup> instead of sucrose, but this substitute was abandoned in studies of dermatitis because the lesions did not develop.

It seemed improbable that the protective action of cornstarch could be due to the starch molecule itself, so an attempt was made to extract the active agent with 95 per cent alcohol. Preliminary studies have shown that this extract is very effective in healing dermatitis, though it does not permit the rats to grow. Furthermore, when extracted starch is substituted for the sucrose of ration 1602, healing does not

TABLE 1

*Preliminary preparation before weaning reduces length of experimental periods*

	NUMBER OF ANIMALS <sup>1</sup>	DERMATITIS		MORTALITY		WEIGHT	
		Average age at onset days <sup>2</sup>	Per cent	Average age at death days <sup>2</sup>	Per cent	Average initial grams	Average final grams
Preliminary preparation <sup>3</sup>	14	54.2 (45-63)	100	67.1 (55-91)	100	26.4	28.4
No preliminary <sup>3</sup> Preparation	18	73.2 (55-98)	94.4 <sup>4</sup>	104.8 (62-182)	100	34.2	44.0

<sup>1</sup> Days from birth.

<sup>2</sup> Screens on fifth day; ration 1545 on fifteenth day; weaned, vitamin supplements on twenty-first day.

<sup>3</sup> Weaned, vitamin supplement on twenty-first day. No preliminary preparation.

<sup>4</sup> One died without developing dermatitis, another had only a mild case.

occur. The observation that the starch extract heals dermatitis, but does not enable the rats to resume growth, is interpreted as evidence that more than one member of the vitamin B complex is affected by irradiation.

5. Thirteen per cent of fat was substituted for an equal amount of sucrose, but when this change was made the results were much less consistent. The appearance of dermatitis was delayed and some died without developing lesions. Two per cent of cod liver oil is used in all rations, but at the worst this does not interfere seriously with the development of dermatitis.

<sup>3</sup> Staley's pure food powdered cornstarch, manufactured by the Staley Mfg. Co., Decatur, Illinois.





As a result of our studies on the constituents of the diet ration 1669 is now used almost exclusively. This differs from ration 1545 only in containing commercial sucrose instead of the recrystallized preparation.

Sherman and Derbigny ('32) stated that the type of dermatitis they were investigating developed much more quickly and was much more severe when the ration was low in protein than when it was high. We compared by our technic a ration that contained 30 per cent of casein with one that contained 10 per cent, and our experience was contrary to that of Sherman and Derbigny. Rats receiving the ration with 30 per cent of protein developed dermatitis more quickly, and survived for shorter periods of time than did those on the low protein diet. This is regarded as additional evidence that the factor with which we are concerned is not the vitamin G of Sherman and of other workers in this field.

An attempt was also made to substitute dried egg white and milk albumin for casein. Only a few animals were studied, but either of these proteins may be used. Dermatitis developed more quickly and the survival periods were shorter when dried egg white was employed, but it is possible that this difference would disappear if the milk albumin had been washed more thoroughly. The various rations we have used are described in table 2, and the results obtained are summarized in table 3.

*Irradiation of solutions.* If the supply of ultraviolet arcs is limited, it is not possible to irradiate sufficient yeast for any considerable number of animals. We have attempted, therefore, to use soluble vitamin B carriers, as it seemed probable that larger quantities could be irradiated effectively in solution than in the dry form. A number of preparations have been studied, but those that seemed most promising were a water extract of yeast and a mixture of equal parts of tikitiki<sup>4</sup> and liver extract.<sup>5</sup> When supplied singly, neither tikitiki nor

<sup>4</sup>Prepared in this laboratory.

<sup>5</sup>We are much indebted to Dr. David Klein, of the Wilson Laboratories, Chicago, Illinois, for generously supplying us with this material.

liver extract is a suitable source of the vitamin B complex. However, the mixture and also the water extract of yeast supported satisfactory growth in controls that received untreated preparations.

When the supplement was irradiated in solution, a volume of 125 cc., containing 15 gm. of solids, was placed in a Pyrex glass baking dish 28 cm. long and 16.5 cm. wide. This dish

TABLE 2  
*Composition of rations<sup>1</sup>*

NUMBER OF RATION	465	1256	1602	1612	1616	1617	1669	1670	1729	1730	1731	1732
Casein 80 <sup>2</sup>	20		20	20								
Casein 180 <sup>2</sup>		20			20	20	20	20	30	10		
Lactalbumin <sup>4</sup>											20	
Ovalbumin <sup>4</sup>												20
Commercial sucrose			71				71		61	81	71	71
Recrystallized sucrose		58			58	58						
Cornstarch	58			71				71				
Crisco						13						
Lard	10	13										
Milk fat					13							
Salts	4	4	4	4	4	4	4	4	4	4	4	4
Cod liver oil	5	2	2	2	2	2	2	2	2	2	2	2
Cellulose	3	3	3	3	3	3	3	3	3	3	3	3

<sup>1</sup> The vitamin B carrier, whether irradiated or untreated, is supplied separately. When the amount is not specified, it will be understood that the total quantity is 100 mg.

<sup>2</sup> Casein 80, extracted with dilute acid.

<sup>3</sup> Casein 180, extracted with dilute acid, with alcohol, and with ether.

<sup>4</sup> Prepared in this laboratory.

<sup>5</sup> Secured from T. M. Duche & Sons, Inc., New York City.

was 14 cm. beneath the mercury arc, and was enclosed on the sides and bottom with a metal jacket through which a stream of tap water was constantly flowing. This jacket was supported on a mechanical rocker so the vitamin solution was in constant motion. The temperature of this solution was never observed to rise above 35°C. Distilled water was added at intervals to prevent undue concentration. Experience indicated this material should be irradiated for 15 hours.

TABLE 8

*Response to irradiated vitamin B carriers is affected by constituents of the diet*

RATION NO.	NUMBER OF ANIMALS AND TYPE OF SUPPLEMENT	DERMATITIS		MORTALITY		WEIGHT	
		Average age at onset days	Per cent	Average age at death days	Per cent	Average initial grams	Average final grams
1602	10—yeast	Casein 80, commercial sucrose				25.6	33.3
		65.9	100	81.2	100		
1669	5—yeast 16—TtL <sup>1</sup>	Casein 180, commercial sucrose				24.5 25.1	32.3 31.6
		52.2	100	77.8	100		
		54.4	100	64.2	100		
1670	8—yeast 4—TtL	Casein 180, cornstarch				22.6 20.2	39.8 43.7
		....	0	121.6	62.5		
		....	0	56.0	25		
465	6—yeast 4—TtL	Casein 80, cornstarch, lard				36.3 34.0	77.6 61.5
		....	0	....	0		
		....	0	97	25		
1612	7—yeast	Casein 80, cornstarch				20.8	59.1
		....	0	66	14.2		
1256	5—yeast 5—TtL	Casein 180, recrystallized sucrose, lard				31.4 20.4	63.6 37.0
		63.0	20	95	20		
		....	0	104	100		
1616	5—yeast 3—TtL	Casein 180, recrystallized sucrose, milk fat 13				31.2 26.3	48.0 40.0
		65.3	60	88.3	60		
		....	0	140.3	100		
1617	7—yeast 5—TtL	Casein 180, commercial sucrose, crisco				30.8 25.6	42.7 45.2
		72.0	42.9	72.5	71.4		
		....	0	160.5	80.0		
1729	8—TtL	Casein 180, 30 per cent, commercial sucrose				26.3	28.7
		46.7	100	60.8	100		
1730	10—TtL	Casein 180, 10 per cent, commercial sucrose				25.9	30.5
		63.0	50	113.2	100		
1731	3—TtL	Lactalbumin, commercial sucrose				27.6	32.3
		56.0	100	100.6	100		
1732	3—TtL	Ovalbumin, commercial sucrose				26.0	27.6
		49.0	100	68.3	100		

<sup>1</sup> Contains equal amounts of dry matter from tikitiki and from liver extract.

Some of our observations on this point are reproduced in table 4, section 2. The controls are shown in section 1.

It seemed possible that the destruction of the antidermatitic factor may not be due to the ultraviolet region of the spectrum, so a few animals were given a preparation that had been irradiated through ordinary window glass. These animals made considerable gains in weight and did not develop

TABLE 4  
*Time necessary to irradiate when supplements are in solution*

NUMBER OF ANIMALS	TIME IRRADIATED, HOURS	DERMATITIS		MORTALITY		WEIGHT	
		Average age at onset days	Per cent	Average age at death days	Per cent	Average initial grams	Average final grams
1. Supplement, dried yeast							
Arc A							
9	10	48.7	100.0	65.4	100.0	28.1	29.5
Arc B							
5	20	56.0	100.0	85.2	100.0	33.6	33.4
2. Supplement, TtL <sup>1</sup>							
6	15	57.4	100.0	69.5	100.0	24.6	34.3
3	10	71.0	33.3	82.0	33.3	25.3	61.3
3	5	....	....	51.0	33.3	25.0	62.0
3. Supplement, TtL, <sup>1</sup> irradiated through glass							
3	15	....	....	....	....	21.0	75.6
Irradiated direct							
3	15	51.3	100.0	65.3	100.0	20.0	32.0

<sup>1</sup> Contains equal amounts of dry matter from tikitiki and from liver extract.

dermatitis. A summary of these observations is included in table 4, section 3.

It was concluded that the mixture of tikitiki and liver extract could be used successfully, but it is not regarded as an ideal preparation for our purpose. The survival periods are no longer than on irradiated yeast, but rats receiving this mixture do not develop the most severe type of dermatitis. The water extract of yeast has not yielded consistent results.

The first preparation seemed ideal as, after irradiation, it produced a severe type of dermatitis. When a second preparation was put into use, practically all the animals died in 4 or 5 weeks without developing lesions of any kind. It was thought this concentrate may have been injured in some manner, presumably by overheating, so it was replaced by a third yeast extract. When this new preparation is irradiated it produces severe dermatitis and, up to date, it is the most satisfactory we have used. There is reason to believe the destruction of the antineuritic factor is relatively slight, probably because the irradiation is conducted at a lower temperature. Our data on this new material are not complete, however, and are not included in this paper. A summary of our observations with dried yeast, the mixture of tikitiki and liver extract, and the water extract of yeast follows in table 5. These observations extended from May, 1932, to December, 1933.

TABLE 5  
*Effect of irradiation on various vitamin B carriers*

SOURCE OF VITAMIN	NUMBER OF ANIMALS OBSERVED	NUMBER OF CASES OF DERMATITIS
Dried yeast	198	190
Tikitiki + liver extract	69	69
Water extract of yeast	86	53

It will be observed that eight rats which received irradiated yeast did not develop dermatitis, but presumably this was because death intervened before there was time for the lesions to develop. None of these animals was more than 69 days old when it died. All of the rats that were given the mixture of irradiated tikitiki and liver extract developed lesions of the skin, but in many cases the symptoms were very mild. It may be that the failure to develop acute symptoms was due to partial destruction of the antineuritic factor, which killed the animals before there was time for the dermatitis to become severe. Approximately half of the animals which received this mixture of supplements were diagnosed as hav-



ing severe polyneuritis, and many of the others had mild cases. It should be mentioned that these symptoms were not regarded as typical, for they seemed identical in every way with those described by Miss Reader ('30). She believes this condition is caused by simultaneous deficiency of B<sub>1</sub> and B<sub>4</sub>. No abnormalities that would suggest polyneuritis were ever observed in rats that received irradiated yeast.

It also developed that the type of lesion now under discussion is healed by tikitiki, at a daily dosage of 100 mg. of dry matter. However, another type developed some weeks later which is apparently identical with the form described by Sherman and Sandels ('31). Since dermatitis has never been ascribed to a lack of vitamin B, we feel the type we have produced may be due to destruction of the B<sub>4</sub> of Miss Reader ('30).

It is now our custom when testing a vitamin supplement for antidermatitic activity by the prophylactic procedure not to use a depletion period. When the curvative method is used, which we prefer, the animals are deprived of all members of the vitamin B complex for the first 7 to 10 days of the experimental period.

*Completeness of destruction due to irradiation.* Studies have also been undertaken to determine whether the antidermatitic factor had been completely destroyed by irradiation. A small number of rats was given more than the minimum dosage we employ in routine studies. It developed at once that destruction was not complete, for when the amount of the supplement was greatly increased dermatitis was delayed, or did not appear at all.

Some effort has been made to estimate the degree of destruction of the antidermatitic factor by determining the minimum amount of untreated yeast that will heal dermatitis. To this end a few rats that had developed mild cases of dermatitis were given graded doses of untreated yeast as the sole source of the vitamin B complex. These observations are summarized in table 6.

It does not seem possible to estimate with any precision just what part of the antidermatitic factor is destroyed by irradiation. The rats that received 60 mg. daily of untreated yeast healed decisively, and those that received 50 mg. improved without complete healing.

It is our opinion that 200 mg. of irradiated yeast is at best no more effective in healing dermatitis than is 50 mg. of untreated yeast. It is estimated, therefore, that about 75 per cent of the antidermatitic substance is destroyed by the technic employed. We are now attempting to devise a procedure that will eliminate this substance completely, and still leave an adequate supply of the antineuritic principle.

TABLE 6  
*Dosage of untreated yeast to heal dermatitis*

NUMBER OF RATS	QUANTITY OF YEAST	REMARKS
	<i>mg.</i>	
3	40	None healed
3	50	Improved
2	60	All healed
2	80	All healed

#### SUMMARY

1. The procedure for producing dermatitis has been slightly modified.

2. Cornstarch contains the factor that prevents dermatitis. The results were less consistent when 13 per cent of fat, hydrogenated cotton seed oil, milk fat, or lard, were included in the ration.

3. For studies of the effect of irradiation on the vitamin B complex, dried yeast is the most suitable carrier that has been examined. Approximately 75 per cent of the antidermatitic activity is destroyed by the technic described.

4. The degree of destruction of the antineuritic factor in dried yeast has not been assayed, but it is not sufficient to interfere with the method.

## LITERATURE CITED

- HOGAN, A. G., AND J. E. HUNTER 1928 The plural nature of vitamin B. J. Biol. Chem., vol. 78, p. 433.
- HOGAN, A. G., AND L. R. RICHARDSON 1932 The effect of ultraviolet rays on the dermatitis preventing vitamin. Mo. Agr. Exp. Sta. Res. Bull. 178.
- OSBORNE, T. B., AND L. B. MENDEL 1919 The nutritive value of the wheat kernel and its milling products. J. Biol. Chem., vol. 37, p. 557.
- READER, V. 1930 Further evidence for a third accessory 'B' factor. Biochem. J., vol. 24, p. 77.
- SHERMAN, H. C., AND I. A. DERBIGNY 1932 Studies on vitamin G (B<sub>2</sub>) with special reference to protein intake. J Biol. Chem., vol. 99, p. 165.
- SHERMAN, H. C., AND M. R. SANDELS 1931 Further experimental differentiation of vitamin B and G. J. Nutrition, vol. 3, p. 395.
- WELLS, A. H. 1921 The preparation of tikitiki extract for the treatment of beriberi. Philippine J. Sci., vol. 19, p. 67. -

# SOME EFFECTS OF THE COMPOSITION OF THE DIET ON THE VITAMIN B AND THE VITAMIN G REQUIREMENT OF THE GROWING RAT<sup>1</sup>

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EIGHT FIGURES

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A previous communication from this laboratory (Guerrant and Dutcher, '33) contained data which suggested that the fiber content of the diet might have a marked effect on the amount of vitamin B complex required by the rat. A review of the available literature at that time revealed that other investigators had made similar observations concerning some of the other constituents of the basal diets commonly employed in vitamin B and vitamin G technics. Most of these observations, however, had been made in connection with the protein and fat content of the diet.

Drummond, Crowden and Hill ('22), Reader and Drummond ('25), Hartwell ('25), Reader and Drummond ('26), Hassen and Drummond ('27), Sherman and Gloy ('27), Hartwell ('28) and Guha ('31) had reported observations concerning various relations of the B complex vitamins to protein utilization. The role played by fats in this connection had been studied by Evans and Lepkovsky ('28, '29) and Guha ('31).

It was difficult to account for the variations in the conclusions drawn by the above investigators. It seemed possible,

<sup>1</sup>Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, March 15, 1934, as technical paper no. 642.

however, that a part, at least, of the lack of uniformity of results might have been due to the fact that vitamin B was not recognized as a complex at the time when some of the experiments were conducted. This fact and, in addition, the wide variations in the composition of basal diets employed by different laboratories in their vitamin B and vitamin G technics, led us to believe that a study of the effects of variations in the composition of the diet on the vitamin B and the vitamin G requirement of the rat could be made with profit.

During the course of this investigation, other communications have been published by Sherman and Derbigny ('32), Evans and Lepkovsky ('32 a, '32 b), Lecoq ('32 a, '32 b, '32 c, '33), Lecoq and Savare ('33), Prunty and Roscoe ('33), Hogan and Pilcher ('33), Francis, Smith and Mendel ('33), Gregory and Drummond ('32), and by Patras and Templeton ('33), which have a bearing on this subject.

The data herein reported give some of the results of a series of experiments carried out during the past 2 years in an effort to gain some insight into the influence of the composition of the diet on the vitamin B and the vitamin G requirement of the growing rat.

#### EXPERIMENTAL

The general plan followed was to feed groups of rats on diets of varied composition, but deficient in both vitamins B and G until early symptoms of vitamin B deficiency appeared; then to supplement such diets with daily dosages of vitamin B concentrate, while continuing the experiment for several weeks until some manifestations of vitamin G deficiency became apparent, at which time further supplementation was effected by daily additions of autoclaved yeast and the animals were continued under observation for several additional weeks.

Both piebald and albino rats were used in the investigation. These animals were placed on experiment in groups of from 5 to 12 animals each, care being taken in their group distribution in order to minimize both litter and sex variations.

Groups of rats, 20 to 21 days old and weighing 39 to 46 gm. each, were placed and maintained in individual cages provided with raised screen grids, and were fed liberal quantities of the diets under consideration. Clean distilled water was kept before the animals at all times. Small additions of iodine were given at weekly intervals. Weekly records were made of the quantity of food consumed, the change in body weight, and the general appearance of each animal. After a preliminary depletion period of 21 days on such diets, each animal was given a measured daily dosage of the vitamin B concentrate during the next 42 to 56 days as a supplement to the diet. At the end of this period, the diet of each animal was further supplemented by weighed daily allotments of autoclaved yeast and the animal was continued under observation for at least 6 additional weeks. Thus all animals which survived the experimental period had existed for 21 days on a diet deficient in both vitamins B and G, followed by 42 to 56 days on the same diet supplemented by daily allotments of vitamin B, and finally for at least 42 days on this diet supplemented by both vitamins B and G.

The vitamin B supplement used in these studies was prepared from dried brewer's yeast. The dry yeast was extracted by percolation with 95 per cent alcohol which had been acidified with concentrated hydrochloric acid (5 ml. of acid per liter of alcohol) as long as the percolate remained colored. The combined percolate was concentrated under reduced pressure until the residue assumed a sirupy consistency. This concentrate was placed in a separatory funnel, an equal volume of water was added, and the mixture was shaken with three successive portions of ether (100 ml. of ether for each kilogram of yeast extracted) in order to remove most of the fatty materials. After partially neutralizing the excess acid with sodium bicarbonate, the concentrate was again reduced to a sirupy consistency by vacuum distillation, and was maintained at a sub-zero temperature for 24 hours. The insoluble materials were filtered off rapidly by means of suction, and sufficient 95 per cent alcohol was

added to make a volume such that 1 ml. of the concentrate represented 10 gm. of the original yeast. This concentrate was kept in the refrigerator during the course of the investigation. A daily dose of 0.1 ml. of this solution was used as a source of vitamin B. Previous tests had shown this quantity to be sufficient to stimulate appreciable growth, when fed to rats receiving a diet complete in other respects.

The vitamin G supplement was prepared from baker's yeast by moistening and dry yeast with a 10 per cent solution of sodium bicarbonate, and then autoclaving the moist mixture for 6 hours at 15 pounds pressure. The autoclaved yeast was later dried and ground to a fine powder. A daily dose of 0.3 gm. of this product was found to supply adequate vitamin G when fed as the only source of this vitamin.

The control diet was similar in composition to that which had previously been used in this laboratory in vitamin B and vitamin G studies. It consisted (in parts per 100) of washed casein 18, salt mixture (McCollum 185) ('18) 4, agar 2, sucrose 20, cod liver oil 2, butter fat 3, and dextrin 51. A diet of this composition was chosen, because it offered possibilities of quite wide variations in its constituents at the expense of the sucrose. This quality was highly desirable, since sucrose, a fairly pure and uniform source of carbohydrate, was selected as the variable compensator in the variations of the other constituents of the diet. This diet alone, and in combination with daily allotments of the vitamin B concentrate, the vitamin G fraction, and a combination of these two preparations, was fed to groups of depleted rats at intervals during the progress of the investigation. These tests were carried out in order to test the adequacy of the daily dosage of the two vitamins, and to show that definite growth responses could be produced by feeding rats a diet of this composition, when it is properly supplemented.

Using a diet of this composition as a basis, five series of diets, in each series of which one of the above ingredients was varied systematically within practical limits, were fed to respective groups of rats during a three-phase experimental period throughout which the animals received 1) neither vita-

min B nor vitamin G, 2) vitamin B, and 3) vitamins B and G. In addition, a sixth series (of four diets), in which 2, 4, 6, and 8 per cent of CellU flour were used as sources of rough-ages instead of agar, was similarly tested. In order to conserve space, the number and composition of the various diets used are presented in table 1.

The components of the various diets were of the quality that is most commonly used in nutritional work of this type. The casein was a good grade South American product. It was extracted with both acidulated water and 95 per cent alcohol until practically free of the vitamin B complex. All ingredients used in the salt mixtures were of the C.P. grade except the ferric citrate and the calcium lactate. These salts were of the U.S.P. grade. The agar was a U.S.P. powder obtained from a reliable chemical supply company. Refined sucrose of the usual commercial grade was used. The Crisco was purchased from a local grocery. The cod liver oil was the E. L. Patch medicinal product. The butter fat was prepared in the usual manner from fresh creamery butter purchased from the college creamery. The dextrin was prepared from commercial cornstarch by moistening the starch with a dilute solution of citric acid and autoclaving for 4 hours at 15 pounds pressure. The CellU flour was a highly purified form of cellulose furnished through the courtesy of the Chicago Dietetic Supply House, Inc. All diets were made up at weekly intervals and were stored in an electric refrigerator.

#### DATA

While close observation was maintained upon all animals during the course of the investigation and a fairly complete record was kept at all times, it is believed that the various differences in growth responses manifested by the several groups of rats receiving the different diets are the most accurate indices of the rat's requirement for vitamin B and for vitamin G while subsisting on these various dietaries. For this reason and also to conserve space, the data obtained in the several series of experiments have been condensed and presented in table 2 and in figures 1 to 8, inclusive.



TABLE 1

*Showing the number and the composition of the various diets used*

DIET NO.	CRISCO	SALTS	CASEIN	AGAR AGAR	CELLU FLOUR	DEXTRIN	SUCROSE	ODD LIVER OIL	BUTTER FAT
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
306 <sup>1</sup>	0	4	18	2	0	51	20	2	3
324	0	4	18	2	0	51	25	0 <sup>2</sup>	0 <sup>2</sup>
319 <sup>1</sup>	0	4	18	2	0	51	20	2	3
320	5	4	18	2	0	51	15	2	3
321	10	4	18	2	0	51	10	2	3
322	15	4	18	2	0	51	5	2	3
323	20	4	18	2	0	51	0	2	3
328	0	1	18	2	0	51	23	2	3
325	0	2	18	2	0	51	22	2	3
329 <sup>1</sup>	0	4	18	2	0	51	20	2	3
326	0	6	18	2	0	51	18	2	3
327	0	8	18	2	0	51	16	2	3
330	0	10	18	2	0	51	14	2	3
331	0	4	8	2	0	51	30	2	3
332	0	4	12	2	0	51	26	2	3
333	0	4	16	2	0	51	22	2	3
334	0	4	20	2	0	51	18	2	3
335	0	4	24	2	0	51	14	2	3
336	0	4	28	2	0	51	10	2	3
337	0	4	18	0	0	51	22	2	3
338 <sup>1</sup>	0	4	18	2	0	51	20	2	3
339	0	4	18	4	0	51	18	2	3
340	0	4	18	6	0	51	16	2	3
341	0	4	18	8	0	51	14	2	3
342	0	4	18	10	0	51	12	2	3
345	0	4	18	0	2	51	20	2	3
346	0	4	18	0	4	51	18	2	3
347	0	4	18	0	6	51	16	2	3
348	0	4	18	0	8	51	14	2	3
353	0	4	18	2	0	0	71	2	3
352	0	4	18	2	0	11	60	2	3
351	0	4	18	2	0	31	40	2	3
350 <sup>1</sup>	0	4	18	2	0	51	20	2	3
349	0	4	18	2	0	71	0	2	3

<sup>1</sup> These diets all had the same composition but were designated under different numbers, since they were used as control diets in different series and at different times.

<sup>2</sup> Crystalline carotene and 10,000 D viosterol were dissolved in petroleum ether and added to the basal diet at weekly intervals in sufficient quantities to furnish 10 Sherman units of vitamin A and 10 A.D.M.A. units of vitamin D for each gram of food.

TABLE 2

Showing the number of animals considered, their average initial weight, their gain in weight, their food intakes, and the approximate caloric equivalent per gram gain in weight

DIET NO.	VARIABLE SUBSTANT	PER CENT VARIABLE CONCENTRATION PRESENT	NUMBER OF ANIMALS CONSIDERED	AVERAGE INITIAL WEIGHT	GAIN DURING DEPLETION PERIOD	GAIN DURING B PERIOD	GAIN DURING B + G PERIOD	TOTAL GAIN IN WEIGHT	WEEKLY FOOD INTAKE DURING DEPLETION PERIOD	WEEKLY FOOD INTAKE DURING B PERIOD	WEEKLY FOOD INTAKE DURING B + G PERIOD	APPROXIMATE CALORIC INCREASE PER GRAM INCREASE IN WEIGHT <sup>1</sup>		
												During depletion period	During the B period	During the B + G period
306	Unsupplemented		9	42	7	17	..	7	23	21	..	42	..	..
306	Vitamin B concentrate		18	41	7	17	..	24	23	23	17	42	39	..
306	Autoclaved yeast		10	41	8	..	9	17	23	..	..	33	..	..
306	Vitamins B and G		9	42	6	..	96	102	23	..	..	46	..	11
324	Fat	0	5	40	-2	14	34	46	16	18	24	..	37	22
319	Fat	5	5	41	3	13	35	51	17	19	26	69	47	26
320	Fat	10	5	42	4	12	44	56	19	16	25	60	50	21
321	Fat	15	5	40	4	12	50	66	19	17	27	64	51	21
322	Fat	20	5	41	4	14	59	77	18	18	31	64	49	21
323	Fat	25	5	40	6	11	48	65	17	16	25	53	58	23
328	Inorganic	1	5	43	-1	20	46	65	20	24	31	..	30	21
325	Inorganic	2	6	42	10	17	34	61	22	23	28	27	33	25
329	Inorganic	4	6	41	9	11	26	46	22	22	24	29	48	28
326	Inorganic	6	6	41	11	8	25	44	21	17	23	23	50	28
327	Inorganic	8	5	42	10	5	23	38	21	16	23	24	74	30
330	Inorganic	10	6	43	6	0	15	21	21	16	22	40	..	44
331	Casein	8	7	41	-3	8	37	42	21	24	27	..	96	19
332	Casein	12	10	41	4	11	42	57	22	27	29	66	79	18
333	Casein	16	9	40	7	13	36	64	22	26	29	38	64	21
334	Casein	20	10	40	8	12	40	60	23	25	28	35	67	18
335	Casein	24	10	41	7	14	38	59	22	24	29	38	55	20
336	Casein	28	6	40	4	16	29	49	20	23	28	60	48	25
337	Agar	0	10	40	4	17	34	55	24	23	29	72	33	21
338	Agar	2	10	41	7	16	36	59	25	25	31	43	38	22
339	Agar	4	9	41	10	22	33	65	26	27	34	31	30	22
340	Agar	6	10	42	13	19	38	70	29	29	35	27	37	24
341	Agar	8	10	41	17	22	33	72	26	28	38	18	31	26
342	Agar	10	10	42	16	23	37	76	25	28	37	19	28	25
345	Cellu flour	2	6	40	7	20	32	54	22	26	31	38	31	24
346	Cellu flour	4	6	41	13	20	33	66	22	27	34	30	33	27
347	Cellu flour	6	6	41	13	22	33	68	23	26	38	21	28	29
348	Cellu flour	8	6	42	19	25	30	74	25	32	36	16	31	30
353	Dextrin	0	4	42	-7	2	21	16	19	15	19	..	181	24
352	Dextrin	11	5	43	-6	2	19	16	19	16	18	..	192	25
351	Dextrin	31	6	41	-6	3	36	33	20	17	23	..	136	17
350	Dextrin	51	6	42	-3	8	41	46	21	19	27	..	57	19
349	Dextrin	71	6	42	5	14	46	65	23	22	31	55	38	17

<sup>1</sup> Calculated on the basis that protein = 4 cal. per gram, carbohydrate = 4 cal. per gram, fat = 9 cal. per gram, and that casein and yeast are 100 per cent protein.

## DISCUSSION

In compiling the above data, records of individual animals which departed abnormally from the group averages were omitted, and only those animals were considered which reacted more or less uniformly as a group. The number of animals which failed to meet this requirement was not large, but did appear to be greater in certain experimental groups. In most cases, these abnormal responses seemed to have followed an excessive period of depletion during the first stage of the experiment. Most of these animals had manifested marked symptoms of beriberi before the end of the 21-day depletion period, and did not make the immediate characteristic response when the daily allotment of the vitamin B concentrate was added. It appears most probable that more uniform growth responses would have been obtained in certain groups of rats had this depletion period been reduced by several days. There is, on the other hand, some evidence which indicates that groups of rats receiving certain diets, could have withstood a more prolonged depletion period without serious consequence.

During the course of the investigation, a total of forty-six animals were used in testing the basal diet and the effectiveness of the vitamin B and the vitamin G fractions in supplementing this diet. The data obtained (fig. 1) show that, a) all rats that received the basal diet unsupplemented died between the twenty-ninth and the fifty-ninth days, b) seventeen of the eighteen rats that received the basal diet supplemented by the vitamin B concentrate lived through the 8-week experimental period, c) nine of the ten animals that received the basal diet supplemented with the vitamin G fraction died before the end of the experiment, and d) those animals that received the basal diet supplemented by both the vitamin B concentrate and the vitamin G fraction made an average gain of 11 gm. per week during the 8-week period.

It was interesting to note in connection with the control diet that, while the composition of the diet remained unchanged from beginning to end, the first animals to die mani-

fed no apparent symptoms of beriberi other than an unsteady gait, and when found dead they were usually clutched to the cage by paws and teeth. Those animals which survived longer, however, manifested very characteristic symptoms several days previous to death. These facts, together with observations made in connection with other experiments, suggest that some relation must exist between the age of the rat and the type of vitamin B deficiency symptoms manifested.

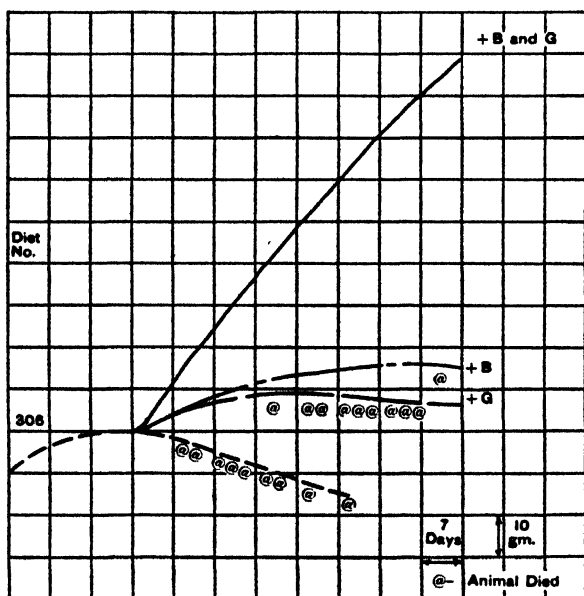


Fig. 1 Showing the effect of supplementing the basal diet (306) with 0.1 ml. of vitamin B concentrate and 0.3 gm. of the vitamin G fraction, separately and in combination.

In a consideration of the data obtained from the groups of rats receiving the diets comprising the fat series (fig. 2), several interesting trends become apparent and seemed to justify some consideration. Diet 324 had been made practically fat free by extracting the properly combined ingredients with ethyl ether for 16 hours in a continuous extractor. Sufficient crystalline carotene and 10,000 D viosterol were added to the ether-free diet to furnish 10 units each of vitamin A

and vitamin D for each gram of food. By using highly potent sources of these vitamins, the fat content of the diet was maintained at the lowest practical level. By increasing the fat content of this diet first by replacing a portion of the sucrose by cod lived oil and butter fat in order to supply the fat soluble vitamins, and then by further replacement of the

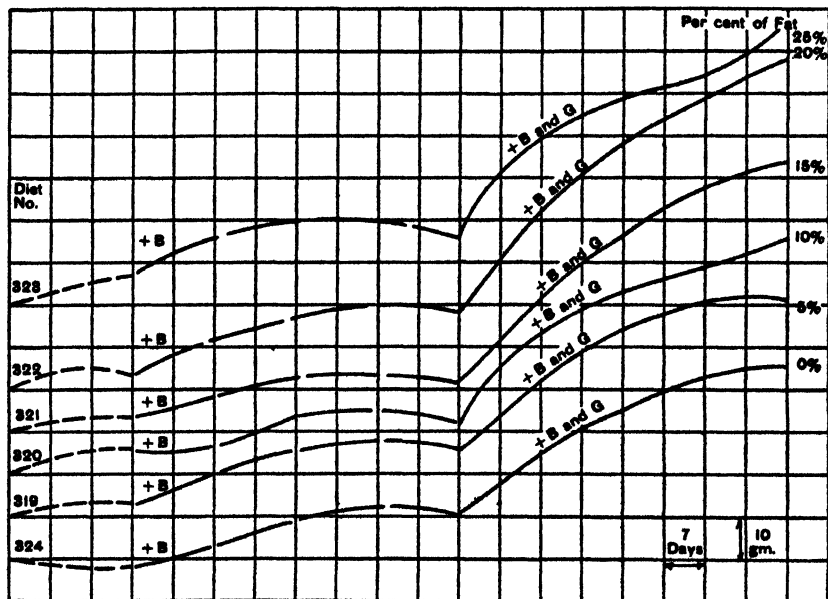


Fig. 2 Showing the average growth responses made by groups of rats receiving 1) vitamin B complex deficient diets of varying fat content; 2) these diets supplemented with daily allotments of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented daily with both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

sucrose with 5, 10, 15 and 20 per cent of Crisco, a series of diets were made up that ranged in fat content from 0 to 25 per cent.

The data obtained through feeding this series of diets to groups of young rats emphasizes three points worthy of note. a) Rats receiving diets containing appreciable quantities of fat (Crisco) were more difficult to deplete of their vitamin B stores than similar animals receiving diets of low fat con-

tent. b) The data do not indicate that the fat content of the diet bears any relation to the rat's requirement for vitamin G. c) When restricted daily allotments of both vitamins B and G were fed to rats, greater growth rates were obtained from those animals receiving diets containing from 15 to 20 per cent of fat than from similar animals receiving corresponding diets of lower fat content.

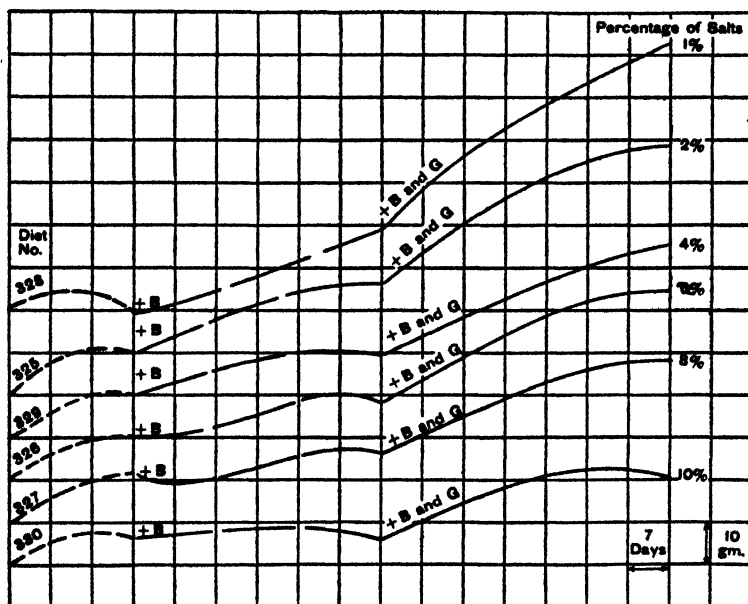


Fig. 3 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying salts content; 2) these diets supplemented with daily dosages of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented with daily dosages of both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

The results obtained with the series of diets in which the salt mixture was the variable constituent under consideration likewise revealed some points of interest (fig. 3). The group of rats which received diet 330 containing 10 per cent of the salt mixture made less than the usual growth response during the 3-week depletion period. In this case the vitamin B concentrate appeared to have little or no supplementing

value. When this diet was further supplemented by autoclaved yeast, the animals grew at a very low rate during the next 5 weeks. During the last 2 weeks that this group of animals was under observation, increased evidence of nutritional failure became apparent. It was highly improbable if any would have lived for 2 additional weeks had the experimental period been extended.

The group of rats receiving diet 327, which contains 8 per cent of the salt mixture, presented a better nutritional picture throughout the experimental period than did those animals receiving the diet containing 10 per cent of this constituent. In fact, as the salt content of the various diets composing the series was decreased through 8, 6, 4 and 2 per cent (diets 327, 326, 329 and 325) more favorable growth responses were obtained. This was also true with diet 328, which contained only 1 per cent of the salt mixture. Frequent observations, however, of the various animals composing the several groups of this series led us to believe that the results which were obtained with this diet are not entirely free of an unsuspected experimental error. At the end of the third week the animals of this group had shown a definite loss in weight, but presented no evidence of beriberi. When the diet was supplemented by vitamin B concentrate, the animals made an immediate growth response. Close observations at this time revealed all animals of the group to be consuming their excreta, especially the urine. This tendency was evidently acquired about the end of the depletion period and was continued throughout the experiment. Consequently, the data obtained on diet 328 are difficult to evaluate in terms of either vitamins B and G or salts intake. The practice of coprophagy was seldom observed among the other groups of animals comprising this series. It is believed, therefore, that the practice in this particular group was an attempt on the part of the experimental animals to maintain a favorable mineral balance while receiving a diet inadequate in inorganic constituents.

When a series of diets, in which the protein constituent (casein) was varied in 4 per cent intervals from 8 to 28

per cent, were fed to groups of rats receiving controlled daily allotments of vitamins B and G, under comparable conditions, no startling differences in growth responses were obtained. (fig. 4). The group of rats which received diet 331 containing 8 per cent of casein lost weight during the first 3 weeks that they were on experiment. While none of the animals

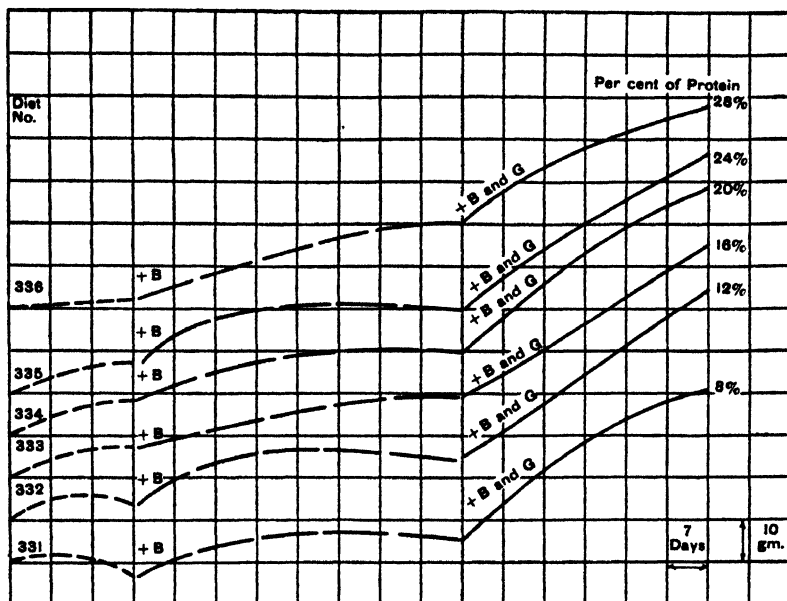


Fig. 4 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying protein content; 2) these diets supplemented daily with 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented by both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

of this group died from beriberi during this time, some of them did exhibit marked paralytic symptoms. Later, three of the ten animals comprising the group were found to be practicing coprophagy; consequently they were removed from the experiment. When a daily allotment of the vitamin B concentrate was given to each animal of this group, a slight gain in weight amounting to 10 gm. resulted during the next 5 weeks, part of which was subsequently lost. The animals



of this group, after being depleted for 3 weeks, and then receiving vitamin B for 8 weeks, did not show any severe symptoms of vitamin G deficiency. There was the usual roughness of hair, scaly feet, and some local depilation of head and body. When the diet was further supplemented with autoclaved yeast, each animal of the group made a very definite growth response and improved markedly in both general appearance and well-being. In this case, it is difficult to determine whether all of this increased growth was due to vitamin G or whether part of it should be attributed to the supplementing effect of the protein added in the form of autoclaved yeast.

When the diet contained 12, 16, 20 or 24 per cent of casein, respectively, as in diets 332, 333, 334 and 335, a definite improvement in growth resulted in all three phases of the experiment when compared to the results obtained on the lower protein diet (331). The group of rats which received diet 336, containing 28 per cent of casein, made only a very slight gain in weight during the first 3 weeks of the experiment. At first there were some indications that this diet was somewhat unpalatable to the young rats. When this diet was supplemented by the vitamin B concentrate, the animals grew at an average rate of 2.5 gm. per week for the next 8 weeks. At this time, the animals of this group had made about the same increase in weight as the groups receiving diets 333, 334 and 335. On further supplementing diet 336 with autoclaved yeast, these animals increased their average rate of growth to 5 gm. per week for the next 6 weeks, which was less than the growth rates of several other groups during this same period.

The data obtained through feeding rats this series of diets indicate that, within the limits of the usual experimental diet, slight difference in protein has no measurable effect upon the rat's requirement for vitamins B and G. When the protein content of the diets is increased or decreased beyond certain limits, other complications arise which make it more difficult to interpret the experimental results obtained.

When a series of six diets, in which agar was the variable constituent, were fed to respective groups of rats, some noticeable differences were observed in the resultant growth responses (fig. 5). The ten animals that received diet 337, which contained no agar, made a slight gain in weight during the first 2 weeks of the depletion period, but part of this gain was later lost during the third and last week of the period.

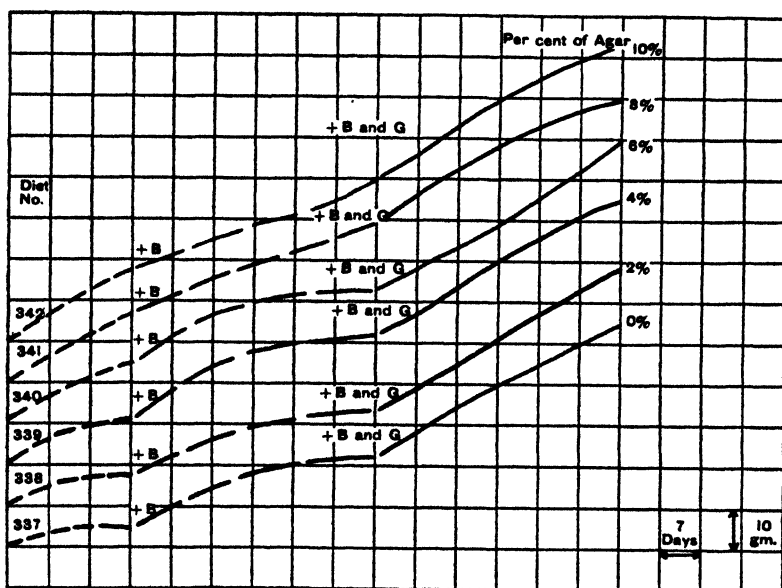


Fig. 5 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying agar content, 2) these diets supplemented with 0.1 ml. daily allotments of the vitamin B concentrate, and, 3) these diets supplemented with both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

At this time all animals of the group appeared to be in an unthrifty condition and some of them showed definite paralytic symptoms. On supplementing this diet with the vitamin B concentrate, all animals reassumed growth for the next 5 weeks. Ruffled fur and retarded growths appeared to be the only visible external symptoms of vitamin G deficiency. On further supplementing the diet with autoclaved yeast, all animals of the group made a uniform and uneventful growth of 5 gm. per week for the next 6 weeks.

When the diet contained 2 per cent of agar (diet 338), the growth response, in general, was very similar to that obtained on the agar-free diet. The only noticeable difference was a smaller loss in weight during the depletion period and fewer symptoms of beriberi during this time. As the agar content of the diets was further increased in 2 per cent intervals, as in diets 339, 340, 341 and 342, marked differences appeared in the responses made by the respective groups of rats during the depletion period. The group of rats receiving a diet containing 10 per cent of agar grew at approximately the same rate as that group which received a diet containing only 8 per cent of this constituent. Those animals which received the diets of higher agar content (6, 8 and 10 per cent) were not only free of beriberi at the end of the 3-week depletion period, but appeared to be in a fair state of nutrition. In spite of marked differences in the growth responses and in the general appearance of the six groups of animals at the end of the 3 weeks, all groups responded quite uniformly when these diets were supplemented, first by the vitamin B concentrate and later by both the vitamin B concentrate and the vitamin G fraction. The results indicate quite conclusively that the uniform addition of vitamins B and G became less effective in increasing the growth rate of the animals as the diets became richer in agar content. This difference in supplementing value was more marked in the case of the vitamin B concentrate.

The above data in themselves were not sufficient to justify the postulation of a specific mechanism by which the vitamin B and the vitamin G requirement of the rat could be affected by the quantity of agar incorporated in the diet. A number of different possibilities suggested themselves, but none appeared tenable without further data. To us the above data appeared to be open to criticism on the ground that an impure or an unrefined agar had been used in the various diets. We anticipated considerable difficulty in attempting to free the agar from impurities by any mode of extraction with vitamin solvents. A more logical plan appeared to be a repetition

of at least part of the experiments involving the agar-containing diets, using CellU flour instead of agar as a source of roughage.

In the CellU flour series, only four diets were fed. Diets 345, 346, 347 and 348 (fig. 6) contained 2, 4, 6 and 8 per cent, respectively, of CellU flour and in other respects were identical in composition to diets 338, 339, 340 and 341 of the agar

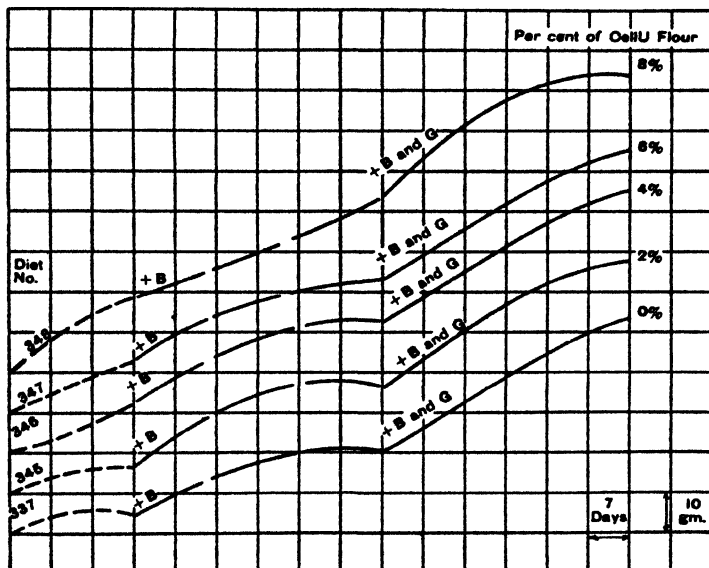


Fig. 6 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying CellU flour content, 2) these diets supplemented by daily additions of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented by daily additions of both 0.1 ml. of vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

series. The test with diet 337 was not repeated at this time. The growth curve presented is the same as presented in figure 5. Four groups of rats of six animals each were fed the CellU flour diets under conditions as nearly identical as possible to that maintained in the agar series. A comparison of figures 5 and 6 will reveal the striking similarity in the two series of results obtained. The nutritional pictures presented by the two groups of rats, one group of which re-

ceived a diet containing a definite percentage of agar, while the other group received a diet containing an equal percentage of CellU flour, were so similar in all respects that a rediscussion appears unnecessary at this time.

While no satisfactory explanation can be offered at this time relative to the physiological mechanism by which the fiber (agar and CellU flour) content of the diet influences the rat's requirement for vitamin B and also vitamin G (but to a less degree), it is hoped that experiments in progress at the present time may shed some light upon the subject.

Unpublished experimental data obtained in this laboratory showed that marked differences in the growth responses of groups of rats receiving identical quantities of vitamin B or vitamin G could be obtained when the respective diets differed only in the kind of carbohydrate. This difference in growth response became quite marked when sucrose-containing diets were compared to dextrin-containing diets. It was believed that by feeding groups of rats a series of diets of constant composition, in which the carbohydrate component was made up of various quantities of sucrose and dextrin, other interesting data would be obtained. In consequence of this fact, a series of six diets was formulated and prepared which ranged in dextrin content from 0 to 71 per cent and in sucrose content from 71 to 0 per cent.

Diet 353 (fig. 7), which contained 71 per cent sucrose and no dextrin, was fed to a group of eight animals. Each animal of the group lost in body weight from the beginning, and by the seventeenth day two of the eight animals were dead. The feeding of the vitamin B concentrate was started at this time, as it was thought that the remaining animals could not survive a 21-day depletion period on the diet and yet retain sufficient vitality for further use in the feeding test. Two more animals died, one on the eighteenth and the other on the twenty-first day. The remaining animals made a definite growth response as the result of receiving the daily allotment of the vitamin B concentrate. The growth-stimulating effect lasted for about 3 weeks when the four surviving animals

began to lose weight gradually for a period of 3 weeks or until the diet was further supplemented by daily additions of autoclaved yeast. During the 2 weeks previous to the addition of the autoclaved yeast, all four of the animals manifested some of the external symptoms usually associated with vitamin G deficiency in the rat. These symptoms include salivation, ulceration of the lining of the mouth and tongue,

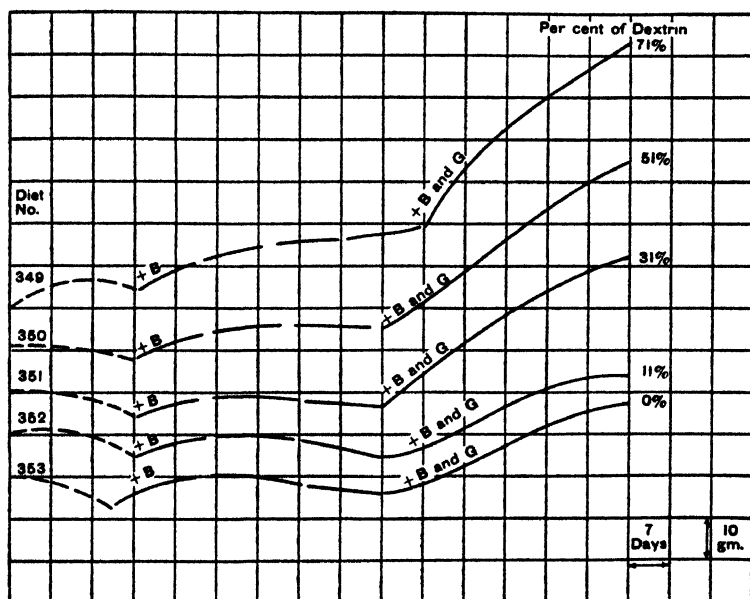


Fig. 7 Showing the average growth responses made by groups of rats receiving, 1) vitamin B deficient diets of varying dextrin content, 2) these diets supplemented by daily allotments of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented with daily allotments of both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

inflamed feet and tail, frequently followed by some signs of necrosis, alopecia areata, and distinct diarrhea. When 0.3 gm. of autoclaved yeast was given to each animal daily, there was some delay in the resumption of growth, but the external symptoms showed marked improvement by the end of the first week. From that point, the animals grew at a fairly uniform rate for the remaining 5 weeks and improved in both activity and general appearance.

On decreasing the sucrose content of the diet to 60 per cent and adding 11 per cent of dextrin as in diet 352, the nutritional picture in general was not greatly improved. The most noticeable difference occurred during the preliminary depletion period. When the sucrose content of the diet was further reduced to 40 per cent and the dextrin increased to 31 per cent, as in diet 351, a distinctly improved nutritional picture was obtained. Further increases of dextrin to 51 per cent and to 71 per cent, as in diets 350 and 349, yielded still greater growth responses and corresponding improvement in the appearance of the experimental animals. Experiments are now in progress which we hope will throw some light upon the mechanism by which the type of carbohydrate exerts its growth-promoting or growth-inhibiting effect, while all other known constituents of the diets are identical as to quality and quantity.

Inspection of figures 2 to 7, inclusive, leads to the general conclusion that a definite correlation exists between the growth responses obtained and the composition of the diets fed. This relationship holds quite well within each of the respective series of diets. But when one compares the results obtained by feeding diets 329, 338 and 350 (table 2 and figs. 3, 5 and 7), this correlation does not appear to be quite so pronounced. These three diets were identical in composition and were fed to three different groups of rats under very similar conditions, except the season of the year in which the experiments were carried out. Diets 306 and 319 were also identical in composition to the above diets but each was fed under different conditions and for this reason the results are not comparable to the results obtained on the above diets.

Consideration of the approximate caloric intake of the various groups of rats during the three phases of the experiments shows that the vitamin B concentrate alone was not always effective in reducing the energy required to produce a unit of growth. In fact, no definite relationship appeared to exist between the quantity of ingested energy and the resultant

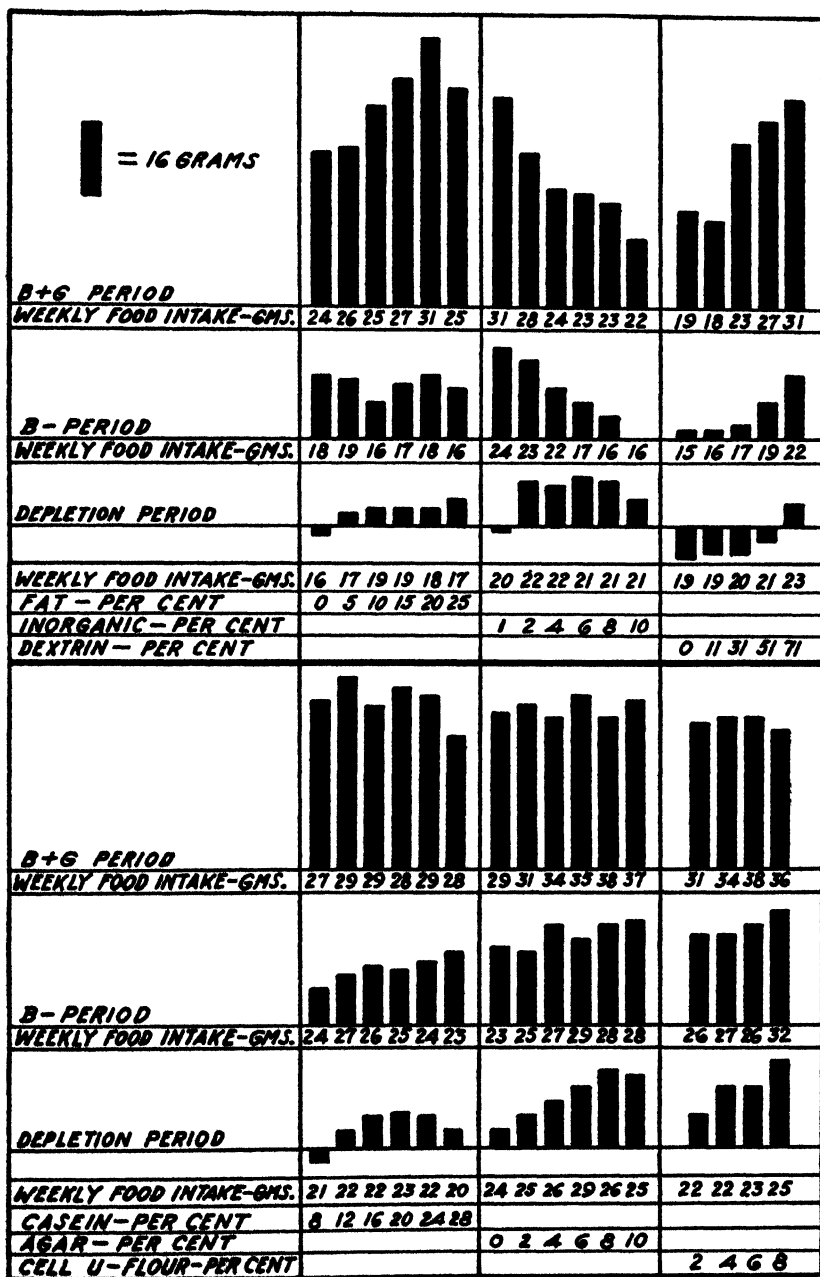


Fig. 8 Showing the relation of the quantity of the various diets consumed to the resultant growth responses made during the three phases of the feeding trials.



rate of growth when groups of young rats were fed the several series of diets, first unsupplemented and then supplemented with a vitamin B concentrate. When these various diets were further supplemented with vitamin G, there was a very noticeable decrease in the quantities of energy required for a unit of growth. The data obtained are insufficient to justify the assumption that vitamin G is unique in its ability to bring about a more efficient utilization of energy for growth. Other essential nutrients would probably be found equally effective in this respect, were they the limiting factors in an otherwise complete diet.

Figure 8 reveals some interesting relationships which were found to exist between the quantity of the various diets consumed and the resultant growth produced. These relationships, as shown among the diets of both the inorganic and the dextrin series, appear to be highly significant.

#### CONCLUSIONS

1. Rats became depleted of their vitamin B reserve somewhat less readily when the basal diet contained increasing amounts of fat. The addition of increasing amounts of fats appeared to have no effect on the vitamin G requirement of the rat. The most satisfactory growth responses (when rats were receiving a somewhat restricted intake of vitamins B and G) occurred when the fat content ranged from 15 to 20 per cent.

2. The rat's requirement for vitamin G is greater as the mineral salts are increased. And, conversely, the lowering of the percentage of mineral salts in the diet seemed to have a sparing effect on this vitamin.

3. No evidence could be obtained to show that variations in the protein content of the diet have an effect on the utilization of vitamins B and G.

4. Increasing amounts of fiber in the form of agar and CellU flour possessed a definite sparing effect on vitamins B and G utilization. The beneficial effect of fiber is thought to be due to the production of more favorable conditions for the growth of microorganisms in the digestive tract.

5. The demand of the rat for vitamins B and G increases when sucrose is fed as the sole source of carbohydrate, while the need for these vitamins decreases as the sucrose is replaced by dextrin.

## LITERATURE CITED

- DRUMMOND, J. C., G. P. CROWDEN AND E. L. G. HILL 1922 Nutrition on high protein dietaries. *J. Physiol.*, vol. 56, p. 413.
- EVANS, H. M., AND S. LEFKOVSKY 1928 Sparing action of fat on the anti-neuritic vitamin. *Science*, vol. 68, p. 298.
- 1929 Sparing action of fat on the antineuritic vitamin B. *J. Biol. Chem.*, vol. 83, p. 269.
- 1932 a The sparing action of fat on vitamin B. *J. Biol. Chem.*, vol. 96, pp. 165 and 179.
- 1932 b The sparing action of fat on vitamin B. *J. Biol. Chem.*, vol. 99, pp. 235 and 237.
- FRANCIS, L. D., A. H. SMITH AND L. B. MENDEL 1933 Growth of rats fed high protein rations supplemented by different amounts and combinations of vitamin B ( $B_1$ ), G ( $B_2$ ) and B-complex. *J. Nutrition*, vol. 6, p. 493.
- GREGORY, E., AND J. C. DRUMMOND 1932 Fat metabolism, with special reference to nutrition on diets devoid of vitamin B. *Z. Vitaminforsch.*, Bd. 1, S. 257. Cited by C. A., vol. 27, p. 2982 (1933).
- GUERRANT, N. B., AND R. A. DUTCHER 1933 Oat hulls as a source of vitamins B and G. *Poultry Science*, vol. 12, p. 373.
- GUHA, B. C. 1931 The physiological function of vitamin B. *Biochem. J.*, vol. 25, p. 1367.
- HARTWELL, G. A. 1925 Vitamin B and the lactating rat's diet. *Biochem. J.*, vol. 19, p. 1075.
- 1928 Protein and vitamin B. *Biochem. J.*, vol. 22, p. 1212.
- HASSAN, A., AND J. C. DRUMMOND 1927 The relation of certain dietary factors in yeast to growth in rats on diets rich in protein. *Biochem. J.*, vol. 21, p. 653.
- HOGAN, A. G., AND R. W. PILCHER 1933 Effects of variations in the amounts of vitamin B and protein in the ration. *Missouri Agr. Expt. Sta. Research Bull.* 195, pp. 1-46.
- LECOQ, R. 1932 a Utilization of protein in relation to the B-vitamins and the equilibrium of the diet. *Bull. Soc. Chem. Biol.*, vol. 14, p. 1067.
- 1932 b Role of the B-vitamins and of the food balance in the utilization of protein. *Compt. rend. Acad. d. sc.*, T. 194, p. 1267.
- 1932 c Are the B-vitamins concerned in the utilization of fats? *Compt. rend. Acad. d. sc.*, T. 195, p. 827.
- 1933 Digestibility and nature of protein as affecting the vitamin B requirement of the pigeon. *Compt. rend. Acad. d. sc.*, T. 196, p. 2033.
- LECOQ, R., AND J. SAVARE 1933 The influence of the constitution of the lipoids on the vitamin B requirement of the pigeon. *Compt. rend. Acad. d. sc.*, T. 196, p. 1693.

- McCOLLUM, E. V., AND N. SIMMONDS 1918 A study of the dietary essential, water soluble B, in relation to its solubility and stability toward reagents. *J. Biol. Chem.*, vol. 33, p. 55.
- PATRAS, M. C., AND R. D. TEMPLETON 1933 The influence of salt intake on the susceptibility of albino rats to diets of low vitamin B content. *Proc. Soc. Exp. Biol. and Med.*, vol. 30, p. 460.
- PREUNTY, F. I. G., AND H. M. ROSCOE 1933 The vitamin B-complex and high protein diets. *Biochem. J.*, vol. 27, p. 699.
- READER, V. B., AND J. C. DRUMMOND 1925 Further observations on nutrition with diets rich in protein. *J. Physiol.*, vol. 59, p. 472.
- 1926 Relation between vitamin B and protein in the diet of the growing rat. *Biochem. J.*, vol. 20, p. 1256.
- SHERMAN, H. C., AND I. A. DERBIGNY 1932 Studies of vitamin G (B<sub>2</sub>) with special reference to protein intake. *J. Biol. Chem.*, vol. 99, p. 165.
- SHERMAN, H. C., AND O. H. M. GLOY 1927 Vitamin B determination and requirement, with special reference to protein intake. *J. Biol. Chem.*, vol. 74, p. 117.

# THE EFFECT OF A HIGH FAT MEAL ON THE RESPIRATORY QUOTIENT AND HEAT PRODUCTION OF NORMAL AND OBESE INDIVIDUALS <sup>1</sup>

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## TWO FIGURES

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Many will agree that not all of the problems concerned with the subject of body weight are settled, although the ideas of some students of obesity are put forth in a manner indicating finality. When accounting for the production of obesity, one finds a difficulty in integrating the various theories as to etiology, abnormal physiology and the role of ductless glands and constitution. The many clinical classifications only add to the confusion.

The concept that body weight depends entirely upon the balance between food eaten and the outflow of energy carries with it the implication that all individuals are potentially thin or fat in accordance with that relationship. Common-place observations make the unqualified acceptance of such a law difficult. If obesity is wholly exogenous, it would be necessary to consume only the equivalent of one patty of butter daily in addition to the needs of the body in order for an individual to become obese in a few years, as has been pointed out by Dubois ('27). Von Noorden stated that the excess consumption of 200 calories daily would lead to the deposition of 7.8 kg. of fat in a year's time.

<sup>1</sup>This work was made possible by the generous gift of the equipment for a metabolism laboratory by the late Mrs. William H. Crosby.

The painstaking quantitative experiments of Newburgh and his co-workers ('30) have shown that the laws of conservation of energy are immutable when applied to the energy balance of obese individuals. Their work ('31) also reflects great doubt upon the 'luxus konsumption' theory of Grafe. They have, also shown, as has been suspected, that the failure of some obese individuals to lose weight while taking a sub-caloric diet, is caused by a temporary hydration of the body. In this sense obesity must be considered exogenous, but the possibility that fat people possess an inherent qualitative metabolic reaction, which is not present in normal individuals, is, we believe, entirely reconcilable with such a concept. Observations on large groups of obese people by Von Noorden, Bauer, and Lyon et al. ('32) show fatness to be a family characteristic in a very large majority of instances. That is, some people may have either as a constitutional or an acquired characteristic, the tendency to convert foodstuffs into fat and store it more readily than others. If the term 'endogenous' be reserved to imply such a reaction, then it would be misleading. Both of these terms, however, are unfortunate as they attempt to differentiate states that are not easily separated clinically, or by laboratory methods.

The storage of small amounts of fat either preformed or converted from carbohydrate has been difficult to measure by short term investigations. Long term observations, which necessitate the measurement of the energy consumed during activity, are quite beyond the patience of either the investigator or the subject. Thus, the question of the nature of obesity has been the target for many opinions and theories which too often have been supported only by a few suggestive observations.

Whether obesity belongs to the so-called constitutional or endocrine diseases, or both, does not essentially alter the problem of its pathologic physiology, unless the clinical types can be clearly separated by some kind of measuring stick. Current opinions concerning this are so controversial and diverse that any discussion of this phase seems impractical.

(Silver and Bauer, '31; Jarløv, '32). In the present study no attempt has been made to make any such differentiation.

The primary purpose of this study was to observe any difference in the metabolic reactions of normal and obese individuals to food. In a preliminary study a few subjects were observed as to the effect of glucose, protein and fat separately. Since one of the first obese subjects studied showed a consistent and a considerable rise in the respiratory quotient after a fat meal, and since the responses to protein and glucose were not dissimilar in the first few normal and obese subjects, it seemed advisable to concentrate our observations on the effect of a fat meal.

The subjects in this study were chosen from both clinic and private practice. The majority were cases of so-called simple obesity; one (J. S.) might be classified as a case of cerebral obesity as her gain followed encephalitis. All observations were made while they were living as usual and taking their accustomed meals. The majority of tests were made at a time when the subjects were out-patients. The weights of the obese subjects varied from 56 per cent to 169 per cent above the average normal—average 81 per cent. In the obese diabetic group the variation was from 14 per cent to 143 per cent above normal—average 47 per cent.

A rest period of at least an hour was allowed. Nearly all of the subjects had previously had one or more metabolism tests and a few had been observed through extended metabolic studies.

The fat meal consisted of 50 gm. of butter on a few bran wafers, which served as carriers, 50 gm. of butter in about 250 cc. of clear chicken broth and 50 gm. of mayonnaise on 50 gm. of lettuce. Fat, 128 gm.; protein, 2.7 gm.; carbohydrate, trace.

This meal was given immediately after satisfactory post-absorptive (14 to 16 hours after food) quotients had been obtained. Absorptive quotients were then determined about each hour for a period of 6 to 8 hours following the ingestion of the fat meal. The subjects were occasionally allowed to

walk to the lavatory, sit up in bed, read and talk after the completion of a period, but were required to rest quietly for at least 20 minutes before the next period. The length of the periods was from 10 to 15 minutes, except in the early stage, when the metabolism of a few subjects was determined for only 6-minute intervals.

The expired air was collected in the Tissot-Boothby Gasometer and analyzed by the Haldane apparatus in duplicate. Subjects whose ventilation volumes were unsteady or whose carbon dioxide percentages in the expired air were erratic, were eliminated. This feature was closely guarded, so that quotients would be unaffected by changes in ventilation.

The Boothby and Sandiford modification of the DuBois standards was used in the calculation of the basal metabolic rates.

*The fasting respiratory quotient.* This varied in the twelve normal subjects (six average and six below average weight) between 0.76 and 0.88 (table 1), the average being 0.825, which corresponds to the usual average post-absorptive quotient stated by DuBois ('27). Joslin ('23) calculated the quotients of 236 of 239 normal individuals and determined the average to be 0.83. Benedict ('19) found the average quotient of sixty-one readings to be 0.795. In the work of Lyon, Dunlop and Stewart ('32) on seven normal, healthy women students, who were accustomed to metabolism tests, an average quotient of 0.797 was found.

In the obese group, twenty subjects, the variation was between 0.72 and 0.83, the average being 0.765. Five were 0.80 or above. Of the eleven obese diabetic patients the quotient varied from 0.73 to 0.785, with only one above 0.80—0.83. The average was 0.767. The diabetes was under control by diet in all of the latter group at the time the observations were made. One was taking insulin, but did not have it on the day of the test. The diets had been about 100 gm. of carbohydrate, a gram of protein per kilogram of body weight, and were designed so the patients would either maintain their weight or lose weight slowly.

TABLE 1  
Normal subjects

Subject	Age	Weight kg.	Height cm.	Sex	B.M.R. per cent.	Basal Period			First Period Av. 70 min.			Second Period Av. 140 min.			Third Period Av. 220 min.			Fourth Period Av. 305 min.			Fifth Period Av. 415 min.		
						O <sub>2</sub> con- sumption cc./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> con- sumption cc./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> con- sumption cc./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> con- sumption cc./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> con- sumption cc./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> con- sumption cc./min.	R.Q.	Cal.s./sq. m./hr.
E.K. 16				F		185	0.85		186	0.85		189	0.81		181	0.82		176	0.88				
H.M. 20	66	175	M		97	249	0.86	40.0	285	0.80	44.9	275	0.80	43.6	285	0.79	41.6	277	0.76	48.4			
H.P. 19	74	177	M		95	283	0.82	59.9	292	0.78	44.2	293	0.80	44.3	299	0.79	45.2						
L.S. 27	77	165	M		96	241	0.86	56.6	271	0.86	45.5	279	0.83	44.3	281	0.78	45.7	280	0.81	41.1	247	0.82	39.2
C.T. 45	77	180	M		102	288	0.79	58.5	500	0.79	44.0	501	0.80	44.2				289	0.75	41.8			
D.G. 23	87	166	F		98	192	0.87	56.5	237	0.75	45.4				229	0.79	42.5	217	0.84	40.7	229	0.77	42.2
F.B. 22	45	150	F		89	168	0.82	53.1	166	0.85	56.0	160	0.84	54.2	169	0.84	56.1						
S.M. 57	46	165	F		92	175	0.85	53.1	197	0.77	57.7	177	0.86	55.1	186	0.86	56.7	168	0.80	56.6	192	0.87	57.9
E.L. 26	50	166	F		96	190	0.76	55.4	196	0.81	57.5	208	0.81	59.3	209	0.79	59.2	218	0.75	40.2			
D.R. 24	48	158	M		90	184	0.84	56.8	202	0.86	40.5	215	0.82	42.4	195	0.79	56.5	222	0.79	45.6			
R.C. 25	51	169	F		107	215	0.80	59.6	225	0.84	41.4	215	0.79	59.4	216	0.75	58.9	240	0.77	48.7			
R.B. 56	45	170	F		91	169	0.84	51.0	161	0.86	51.5	175	0.77	55.1	179	0.85	54.9	188	0.78	56.2	195	0.76	57.1



*The effect of the fat meal on the respiratory quotient.* Of the group containing six of average weight and six who were below (i.e., 'normal') there was no essential difference in the response to a fat meal. There was no change in the average respiratory quotient at the end of an hour. At the second hour the respiratory quotient was depressed (average 0.80), where it remained to the fifth hour. At the sixth hour there was a beginning trend upward, suggesting a return to the fasting level (average 0.825); unfortunately, the subjects were not observed long enough to demonstrate this point.

In the obese, non-diabetic group the respiratory quotients rose steadily from an average of 0.765 to 0.805 at the end of two hours dropping to 0.79 at the fourth hour (table 2). Only six of the group were observed through the eighth hour; then, the average quotients had returned to the average fasting level.

The average fasting quotient of the obese-diabetic group (table 3) was nearly the same as the non-diabetic group. The fat meal produced a slightly upward trend to 0.78 with a return to the fasting value in 5 hours. This curve was similar to the non-diabetic group, except that its rise was less and its fall came sooner.

*The specific dynamic action of the fat meal.* The total extra calories during absorption and assimilation of fat was essentially the same in the obese and normal groups, 25.25 and 26.15 calories, respectively, during a 5-hour period. The specific dynamic action based on surface area was, however, 30 per cent greater in normals. In the obese-diabetic group the total extra calories were 23 per cent higher than the average of the other groups. This effect of the meal in all groups was still evident at the end of the period of observation, even though the respiratory quotients had returned to the fasting levels in two of the groups, obese and obese-diabetics.

The specific dynamic action of a fat meal in the six individuals comprising the thin, normal sub-group was not significantly different from those of average weight, or those who

TABLE 2  
*Obese subjects*

Subject	Age	Weight kg.	Height cm.	Sex	B.M.R. per cent.	Basal Period			First Period Av. 90 min.			Second Period Av. 170 min.			Third Period Av. 250 min.			Fourth Period Av. 350 min.			Fifth Period Av. 400 min.		
						O <sub>2</sub> consumption lit./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> consumption lit./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> consumption lit./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> consumption lit./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> consumption lit./min.	R.Q.	Cal.s./sq. m./hr.	O <sub>2</sub> consumption lit./min.	R.Q.	Cal.s./sq. m./hr.
J.R.	40	105	156	F	111	279	0.77	59.2	515	0.77	44.5	520	0.79	45.1	526	0.81	46.1	515	0.80	44.2	520	0.84	45.6
M.S.	48	105	152	F	87	213	0.78	50.5	220	0.83	52.2	234	0.82	53.8	244	0.81	55.5	241	0.76	54.4			
S.J.	53	118	161	F	91	276	0.84	57.2	292	0.78	58.7	292	0.78	58.7	282	0.82	57.8	290	0.80	58.7			
H.P.	56	109	161	F	109	284	0.78	59.0	283	0.82	59.1	275	0.80	57.9	296	0.76	40.4	286	0.85	59.8	265	0.77	55.9
A.C.	18	103	166	F	95	261	0.75	55.4	295	0.84	40.7	259	0.82	55.8	277	0.85	58.4						
B.T.	45	100	153	F	97	252	0.74	55.8	261	0.79	58.5	269	0.80	59.6							276	0.74	40.1
K.S.	51	89	153	F	95	222	0.80	54.5	250	0.82	58.9	240	0.80	57.0	252	0.79	58.8	262	0.79	40.2	256	0.80	59.5
S.C.	51	106	166	F	107	275	0.73	56.9	296	0.78	40.2	295	0.78	40.5	500	0.74	40.5	515	0.72	41.8			
F.K.	17	107	172	F	97	275	0.83	56.4	285	0.76	56.9	297	0.77	58.6	275	0.80	55.8	280	0.78	56.4			
M.C.	17	97	164	F	90	248	0.75	55.5	248	0.82	55.8	254	0.80	54.6	257	0.78	54.7	255	0.79	54.5	249	0.80	55.8
E.J.	56	126	167	F	101	296	0.74	56.5	500	0.78	57.2	519	0.78	59.6							484	0.78	58.8
J.S.	57	95	157	F	100	255	0.82	56.0	287	0.82	40.1	259	0.77	58.5	256	0.78	58.0	269	0.78	59.9			
J.A.	168	151	F			535	0.77	55.9	550	0.81	58.8	561	0.74	41.6	598	0.76	45.1						
M.T.	55	102	170	F	91	250	0.72	55.1	261	0.76	55.0	281	0.79	57.9	254	0.78	54.1	264	0.79	55.6	265	0.79	55.8
L.O.	54	125	167	F	98	279	0.77	55.6	294	0.79	58.5	296	0.77	57.7	514	0.79	59.9						
E.R.	26	118	174	F	92	275	0.72	55.7	299	0.79	57.9				511	0.75	58.4						
L.O.	45	154	174	M	122	586	0.76	46.0	429	0.77	50.6	404	0.82	48.1	598	0.82	47.4	408	0.78	48.1	415	0.79	48.9
G.R.	26	88	151	F	96	255	0.72	56.0	288	0.76	56.8	248	0.83	59.1	251	0.76	58.6	241	0.77	57.5	254	0.79	59.5
F.B.	22	119	160	F	96	266	0.82	55.4	279	0.82	57.1	274	0.84	56.7	282	0.80	57.5	262	0.86	55.2			
C.J.	45	99	161	F	105	256	0.76	56.5	251	0.82	56.0	251	0.80	55.8	266	0.80	57.9	254	0.78	56.1			

TABLE 3  
*Obese diabetic patients*

Subject	Age	Height in.	Weight lb.	Sex	B.M.R. per cent	Basal Period			First Period Av. 75 min.			Second Period Av. 145 min.			Third Period Av. 230 min.			Fourth Period Av. 325 min.			Fifth Period Av. 430 min.		
						Op gon- ad- renal	N.Q.	Cal. m./sq. hr.	Op gon- ad- renal	N.Q.	Cal. m./sq. hr.	Op gon- ad- renal	N.Q.	Cal. m./sq. hr.	Op gon- ad- renal	N.Q.	Cal. m./sq. hr.	Op gon- ad- renal	N.Q.	Cal. m./sq. hr.	Op gon- ad- renal	N.Q.	Cal. m./sq. hr.
M.W. 64	75	182	F	100	215	0.79	54.1	240	0.79	57.6	233	0.79	57.4	249	0.75	59.4	256	0.79	59.9				
G.S. 49	89	160	F	99	222	0.78	54.5	250	0.80	54.8	245	0.78	56.9	250	0.79	57.7	257	0.79	58.9				
M.M. 52	81	156	F	97	224	0.76	55.1	232	0.82	56.8	247	0.79	59.0	252	0.79	59.8							
A.K. 40	108	177	M	105	320	0.75	40.2	335	0.78	45.0	345	0.79	45.6	356	0.80	45.5							
M.D. 68	67	162	F	106	205	0.74	55.5	211	0.79	56.9	213	0.79	57.5	224	0.75	58.8	250	0.71	45.1				
J.M. 59	99	159	F	102	240	0.79	54.7	265	0.78	58.4	272	0.76	59.2	255	0.79	56.9	249	0.79	58.9				
W.R. 49	86	175	M	81	259	0.74	54.4	280	0.78	57.4	272	0.77	56.4	286	0.78	58.5	286	0.76	58.1				
L.B. 44	150	160	F	128	374	0.85	45.4	422	0.79	50.7	436	0.77	52.0				401	0.80	48.5	416	0.76	59.0	
A.C. 60	84		F		218	0.76		259	0.75		262	0.81		253	0.80		251	0.72					
M.B. 40	82	160	F	96	216	0.79	55.7	240	0.78	57.5	239	0.76	57.0	256	0.81	40.2	279	0.72	45.8				
B.D. 35	85	154	F	76	175	0.75	27.5	200	0.76	51.6	201	0.77	52.0	196	0.76	51.0	192	0.79	50.5				

were obese. These individuals, however, although below the average weight, were not as thin as those studied by Mason ('27), upon whom he found an increased specific dynamic action of a fat.

*The basal metabolic rate.* This was within the average accepted normal in all groups with these exceptions: L. B., an extremely obese diabetic who always showed an increased rate (four determinations on separate days). B. D., a diabetic, who had recently recovered from an infection, showed a low rate as would be expected. L. O., whose rate was plus 22 per cent, was a plethoric gourmand.

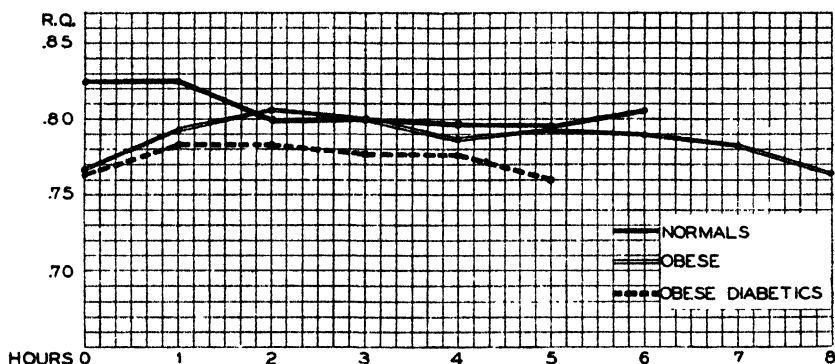


Fig. 1 Composite course of the respiratory quotient after fat meal; normal, obese, and obese diabetic individuals.

#### DISCUSSION

The most significant finding in this study is, we believe, that the post-absorptive respiratory quotient in the obese group was lower than that of the normal (fig. 1). Hagedorn ('27, '28) has reported the same result in a similar study of obese and normal subjects who had previously ingested a high carbohydrate diet. The quotients were higher than those obtained by us presumably because of this diet, but the average difference was the same—0.05. The explanation of this cannot readily be shown by direct observations, but it does permit of some attempt at analysis, as Hagedorn has done.

Lyon, Dunlop and Stewart ('32) also found the average of thirty-four observations on the respiratory quotient of obese subjects on a diet of 2500 calories (hospital maintenance diet) to be 0.755. The majority of the patients lost weight on it, so the authors considered it to be slightly sub-caloric.

Means ('15) published the metabolism data of eighteen obese subjects. Four of these he had studied himself, while fourteen were collected from the literature. The average post-absorptive respiratory quotient of his subjects was 0.755, whereas the others were 0.78.

The obese person may possess a mechanism by which he converts carbohydrate to fat with greater ease than the normal, so that the food mixture undergoing combustion in the post-absorptive state contains a relatively smaller amount of carbohydrate. The observations of Krantz and Means ('27) on the behavior of the respiratory quotient after the administration of epinephrin to normal and obese subjects, lends further support to such a hypothesis. It is ordinarily assumed that epinephrin causes a discharge of the more readily available carbohydrate in the expenditure of the extra calories required by its presence. These observers found that the average rise of the quotient was 19.6 per cent in normal individuals, whereas in the obese group it was 11.2 per cent. These observations were made when the subjects were in a post-absorptive state and it seems plausible that the failure of the quotient of the obese subjects to parallel the normal was because more of the carbohydrate, which had been previously ingested by the obese, had been converted to fat, rather than stored as such.

It is difficult to harmonize the above statements, however, with the fat-absorptive quotients that our experiments show. These would seem to indicate that both normal and obese use about the same food mixture after the ingestion of fat and imply that during this process at least the obese burns just as much carbohydrate as the normal. That there may be a carbohydrate-fat or fat-carbohydrate conversion at that stage, which is not reflected in the respiratory quotient, is unlikely,

unless it is compensated for. The more probable explanation would seem to be that fat requiring a certain amount of carbohydrate for its perfect combustion commands a release of carbohydrate from storage. It may be supposed that the obese diabetic's storehouse of carbohydrate is reduced, thereby accounting for the lesser rise after the ingestion of fat and, also, the more rapid fall to the base line. Our results are at variance with those of Wang, Strouse and Saunders ('25), who observed the effect for 5 hours of a fat meal on four obese subjects. The average fasting quotient in this group was 0.83 (non-protein) and the effect of the meal produced a downward trend of the quotient to 0.76 (average). Although the authors do not specifically state, it is assumed that these subjects had previously ingested an ordinary diet. The fat meal consisted of "fat varying from 55 to 126 grams in the form of slightly sweetened ice cream made from 40 per cent cream."

Attempts have been made by many investigators to explain obesity on the basis of a reduced specific dynamic action of food. This might be significant if the obese had little or no specific dynamic action in contrast to thin people. But, as Peters and Van Slyke ('31) point out, it could account for but 3 to 5 per cent of the total metabolism for the day. Evidence of a lowered specific dynamic action in the obese was largely based upon the work of Rolly ('21), Plaut ('23), and Wang, Strouse and Saunders ('24). More recent and extensive work by Lauter ('26), DuBois ('29), Strang and McClugage ('31), and Johnston ('32) throws a great doubt on there being any great difference in the specific dynamic action of food in the obese and normal person.

Although this study concerns the metabolism of fat only, no essential difference was found in the specific dynamic action in the obese and in the normal, when based upon total extra heat produced (fig. 2). For a 5-hour period the average obese spent 25.25 and the average normal 26.15 extra calories. So it appears that the total extra heat produced by fat absorption and assimilation is in both cases the same, but is distributed

over a greater cellular mass in the obese than in the normal. Strang and his co-workers ('31) observed a similar effect using a diet containing protein, 40 gm.; carbohydrate, 52 gm.; and fat, 26 gm. They believe that this extra heat produces a greater physiologic strain in the thin person, but does not necessarily represent a greater specific dynamic action as far as total calories are concerned.

In our normal group the absorptive quotients were depressed from the fasting level after the fat meal, while the opposite effect was found in the obese subjects. In the obese diabetics the absorptive quotients changed little, even though

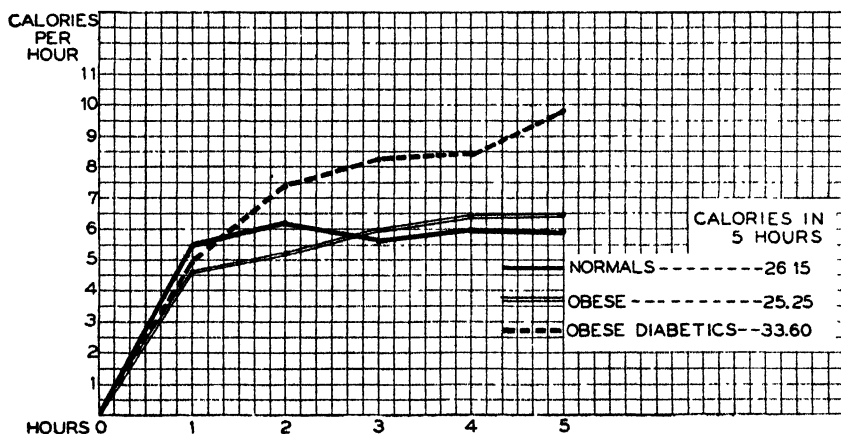


Fig. 2 Composite course of specific dynamic action (calories above basal) after fat meal; normal, obese, and obese diabetic individuals.

this group showed the greatest specific dynamic action. This is commented upon because of the work of Krogh and Lindhard ('20), who found that increase of metabolism caused by physical activity was accompanied by a lower quotient if the post-absorptive quotient was high, and a higher quotient if the post-absorptive quotient was low. Our results suggest that the same phenomena may take place if the increase in metabolism is caused by the specific dynamic action of fat. However, it will be seen that at the end of our observations the specific dynamic action was still present, although the respiratory quotients were returning to their respective fasting levels; this is particularly true in the obese diabetic subjects.

## SUMMARY

1. The possibility that a qualitative metabolic variation exists in the obese person is discussed. This variation from the normal is based upon the finding of a lower post-absorptive respiratory quotient in the obese subject.

2. The effect of a large fat meal has been observed on twelve normal subjects, six of whom were slightly underweight, twenty non-diabetic obese and eleven obese diabetic subjects. The absorptive respiratory quotients indicate that the food mixture during this absorption is approximately the same in normal and obese persons. This response in the diabetic obese group suggests that less carbohydrate was being combusted during the utilization of fat. The observations were extended long enough to demonstrate the returning trend of the respiratory quotient to the post-absorptive level. No correlation between the curves of the absorptive respiratory quotients and the curves of the specific dynamic action could be demonstrated.

3. A specific dynamic action of fat was present in all groups. It was found to be approximately the same in the obese and the normal people. This action, however, was considerably greater in the obese diabetic individuals, which we have made no attempt to explain.

## LITERATURE CITED

- BAUER, J. 1931 Quoted from Silver, S., and J. Bauer. *Am. J. Med. Sci.*, vol. 181, p. 772.
- BENEDICT, F. G. 1919 Quoted from Lyon, D. M., D. M. Dunlop, and C. P. Stewart. *Ibid.*, p. 1109.
- DUBOIS, E. F. 1927 *Basal metabolism in health and disease*. Lea & Febiger, Philadelphia, p. 235.
- 1927 *Ibid.*, p. 249.
- DUBOIS, E. F., H. J. SPENCER, W. S. MCCLELLAN, AND E. FALK 1929 The specific dynamic action of protein in the obese. *J. Clin. Invest.*, vol. 7, p. 499. (Soc. proc.)
- HAGEDORN, H. C. 1928 Problems in the metabolism of obesity. *Acta med. Scandinav.*, supp. 26, p. 154.
- HAGEDORN, H. C., C. HOLTEN, AND A. H. JOHANSEN 1927 The pathology of metabolism in obesity. *Arch. Int. Med.*, vol. 40, p. 30.



- JARLØV, E. 1932 The clinical types of abnormal obesity. *Acta med. Scandinav.*, supp. 42.
- JOHNSTON, M. W. 1932 The specific dynamic response to protein of individuals suffering from disease of the hypophysis. *J. Clin. Invest.*, vol. 11, p. 437.
- JOSLIN, E. P. 1923 Quoted from Lyon, D. M., D. M. Dunlop, and C. P. Stewart. *Ibid.*, p. 1109.
- KRANTZ, C. I., AND J. H. MEANS 1927 Epinephrin reaction in obesity. *J. Clin. Invest.*, vol. 3, p. 565.
- KROGH, A., AND J. LINDHARD 1920 The relative value of fat and carbohydrate as sources of muscular energy; with appendices on the correlation between standard metabolism and the respiratory quotient during rest and work. *Biochem. J.*, vol. 14, p. 290.
- LAUTER, S. 1926 Zur Genese der Fettsucht. *Deutsches Arch. f. klin. Med.*, Bd. 150, S. 315.
- LYON, D. M., AND D. M. DUNLOP 1932 Treatment of obesity; comparison of effects of diet and thyroid extract. *Quart. J. Med.*, vol. 1, p. 331.
- LYON, D. M., D. M. DUNLOP, AND C. P. STEWART 1932 Respiratory quotient in obese subjects. *Biochem. J.*, vol. 26, p. 1107.
- MASON, E. H. 1927 Abnormal specific dynamic action of protein, glucose and fat associated with undernutrition. *J. Clin. Invest.*, vol. 4, p. 353.
- MEANS, J. H. 1915 Studies in the metabolism of obesity and pituitary disease. *J. Med. Research*, vol. 32, p. 121.
- NEWBURGH, L. H., AND M. W. JOHNSTON 1930 Endogenous obesity—A misconception. *Ann. Int. Med.*, vol. 3, p. 815.
- PETERS, J. P., AND D. D. VAN SLYKE 1931 Quantitative clinical chemistry. Interpretations. Williams & Wilkins Company, Baltimore, p. 36.
- PLAUT, R. 1923 Gaswechseluntersuchungen bei Fettsucht. *Deutsches Arch. f. klin. Med.*, Bd. 142, S. 266.
- ROLLY, F. 1921 Metabolism in obesity. *Deutsche med. Wehnschr.*, Bd. 47, S. 887.
- SILVER, S., AND J. BAUER 1931 Obesity, constitutional or endocrine. *Am. J. Med. Sc.*, vol. 181, p. 769.
- STRANG, J. M., AND H. B. McCLUGAGE 1931 The specific dynamic action of food in abnormal states of nutrition. *Am. J. Med. Sc.*, vol. 182, p. 49.
- VON NOORDEN Quoted from DuBois, E. F., p. 235.
- VON NOORDEN Quoted from Silver, S., and J. Bauer, p. 772.
- WANG, C. C., S. STROUSE, AND A. D. SAUNDERS 1925 The metabolism of obesity. IV. The distribution of energy production after food. *Arch. Int. Med.*, vol. 36, p. 397.
- 1924 Studies on the metabolism of obesity. III. The specific dynamic action of food. *Arch. Int. Med.*, vol. 34, p. 573.
- WILEY, F. H., AND L. H. NEWBURGH 1931 The doubtful nature of 'Luxuskonsumption.' *J. Clin. Invest.*, vol. 10, p. 733.

# CELLULOSE IN THE DIET OF RATS AND MICE

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## TWO FIGURES

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Since the literature upon cellulose as a dietary constituent has been well reviewed recently by Rose ('32), we are including no review in this report. In a preliminary report (McCay, '29) we discussed the effect of feeding purified cellulose at very high levels to rats. Ordinary washed cellophane was used as a source of pure cellulose, since its physical composition is much more satisfactory for feeding animals than such substances as filter paper. Contrary to our expectations, rats fed diets containing 10 to 20 per cent cellulose were able to attain maturity, although the growth rate proved slower in these trials than that for animals in the colony fed a stock diet.

In order to determine the effect of this high cellulose<sup>1</sup> diet upon the life span of rats, we fed two groups upon the following diets: no. 4, casein 25, salt mixture 4, lard 20, sugar 36, cellulose 10, vitavose 5 and cod liver oil 3; no. 7, casein 25, salt mixture 4, lard 20, sugar 26, cellulose 20, vitavose 5 and cod liver oil 3. Eight male rats were placed upon diet 4 at

<sup>1</sup> In all cases in which we have employed pure cellulose in our diets it has been in the form of regenerated cellulose washed free from glycerine and ground finely. Most of this cellulose has been furnished through the courtesy of the Sylvania Corporation, New York City, and is the product marketed under the trade name of 'Sylphrap,' except that the compound fed has been washed free of glycerine. 'Regenerated cellulose' is the term commonly used in industry meaning cellulose purified by solution and reprecipitation.

the time of weaning. They attained a mean age of  $674 \pm 35$  days. Nine male rats were fed diet 7 during the same period. They attained a mean age of  $602 \pm 61$  days. For comparison we have shown in figure 1 the mortality curve for seventy-five male rats which were fed the stock diet used in our colony and previously described by Maynard ('30). The mean length of life for male rats upon this diet proved to be  $503 \pm 12$ . This stock diet has proved satisfactory for growth and reproduction for a number of years, but it is not satisfactory for longevity. The life span upon the lower cellulose level exceeds that found by Campbell ('28), upon her best diet. From

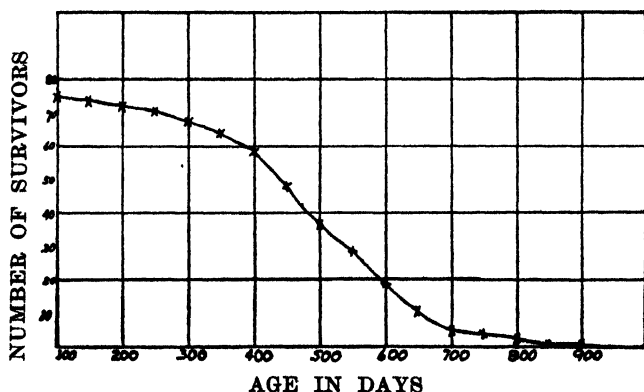


Fig. 1 Mortality curve for male rats fed a stock diet that allowed good growth and reproduction.

a consideration of the meager data of Slonaker ('12), it is evident that life spans of 600 odd days involve only middle life and that the life span for a rat must be well over 1000 days if we are to discuss it under the term of longevity in its usual meaning. There can be little doubt that such high cellulose diets are abnormal for Omnivora. The life span is not shortened, however. The longer life span in the present case of high cellulose diets probably has no relation to the cellulose, but is the result of the slow growth, as we have postulated elsewhere ('33).

In order to determine the influence of other inert materials upon the rate of growth the diets of table 1 were designed.

These diets all included a large amount of fat in order to determine whether the slower growth rates in the preceding experiments were due to the inability of the animal to ingest sufficient calories in the presence of large amounts of cellulose.

Table 2 contains the weights of rats at the start and after 12, 24 and 36 weeks for the animals upon each of these diets. In this case the better growth was made by the animals upon the diets containing the cellulose and agar, possibly indicating a better tolerance for the high calory diet in the presence of inert material. The growth rate was not improved over that previously found for diets 4 and 7, however. This indicated the animal body had ample capacity to ingest a considerable

TABLE 1  
*Diets for rats containing cellulose and agar-agar*

NUMBER	8	9	10
Dry liver	25	25	25
Wheat germ	5	5	5
Cellulose	20	—	—
Agar-agar	—	20	—
Salt mixture	5	5	5
Sugar	—	—	20
Lard	40	40	40
Cod liver oil	5	5	5

TABLE 2  
*Mean weight and probable error of mean weight at start and after 12, 24 and 36 weeks feeding rats diets varying in inert materials*

DIET NUMBER	NUMBER RATS AND SEX	MEAN WEIGHT, GM., BEGINNING	MEAN WEIGHT, GM., AFTER 12 WEEKS FEEDING	MEAN WEIGHT, GM., AFTER 24 WEEKS	MEAN WEIGHT, GM., AFTER 36 WEEKS	
8	4 ♂	43.0 ± 1.61	148.8 ± 10.29	205.3 ± 15.53	225.7 ± 11.70	High cellulose
8	6 ♀	38.8 ± 0.504	148.2 ± 5.81	223.3 ± 11.20	227.2 ± 13.60	High cellulose
Total	10 (♂ + ♀)	40.5 ± 0.80	148.4 ± 5.04	216.1 ± 8.86	226.7 ± 8.07	High cellulose
9	4 ♂	36.3 ± 2.50	145.8 ± 3.51	217.3 ± 8.59	275.0 ± 9.68	High agar
9	6 ♀	30.5 ± 2.00	139.3 ± 3.83	211.3 ± 9.50	240.3 ± 16.68	High agar
Total	10 (♂ + ♀)	32.8 ± 1.52	141.9 ± 2.65	213.7 ± 6.23	251.0 ± 10.97	High agar
10	4 ♂	47.0 ± 1.67	104.8 ± 9.19	187.5 ± 13.04	248.8 ± 6.81	No roughage
10	6 ♀	49.0 ± 1.00	88.0 ± 5.63	165.5 ± 6.70	220.8 ± 7.79	No roughage
Total	10 (♂ + ♀)	48.2 ± 0.86	94.7 ± 5.04	174.3 ± 6.60	232.0 ± 6.00	No roughage

excess of inert materials. These animals were maintained upon the diets for 9 months. They slowly attained a weight of 300 gm., but few exceeded it.

In these experiments no differences were observed between the agar and cellulose. The feces of all animals upon these diets were large, solid and light in color.

In the meantime, from many observations in the course of various experiments, the value of vitavose or wheat germ as a supplement to furnish adequate B factors had been doubted. For this reason the last experiments were repeated with new sources of vitamin B. The composition of these diets is shown in table 3.

TABLE 3  
*Second series of diets containing agar-agar and cellulose*

NUMBER	27	8-A	9-A	10-A
Dry liver	25	25	25	25
Yeast	5	5	5	5
Cellulose	—	20	—	—
Agar-agar	—	—	20	—
Salt mixture	5	5	5	5
Sugar	45	—	—	20
Lard	15	40	40	40
Cod liver oil	5	5	5	5

These diets were designed to compare the growth rates of rats upon high roughage diets containing large amounts of fat with one without the roughage. Diet 27, containing a moderate amount of fat, was inserted, since earlier work had indicated a slower growth rate upon a high fat diet. In these series eight male rats were used for each diet. They were reared in the usual manner in cages with false bottoms.

The growth rates, summarized in table 4, upon diets 27, 8-a, 9-a and 10-a were the same. This indicates that in the presence of an adequate amount of yeast and with adequate calories the rat can consume enough food for optimum growth requirements even in the presence of large amounts of inert materials, such as cellulose or agar. The high intake levels of purified cellulose and agar-agar seem to give much the same results as far as growth and well being of animals can be observed from external appearances.

The rat can tolerate 20 per cent of inert material in its diet if the other ingredients furnish adequate vitamins. Optimum growth can be obtained with this high roughage diet. Earlier failures were due to inadequate vitamins.

*The tolerance of mice for high roughage diets.* Since it was found that rats could tolerate high levels of cellulose we desired to study another related species.

TABLE 4

*Mean weights of male rats at the beginning and at 12 and 24 weeks of feeding diets with and without roughage. No differences in growth were found*

DIET NUMBER	NUMBER RATS AND SEX	MEAN WEIGHT BEGINNING	MEAN WEIGHT AFTER 12 WEEKS FEEDING	MEAN WEIGHT AFTER 24 WEEKS	
		gm.	gm.	gm.	
8a	8 ♂	43.5 ± 1.59	198.4 ± 3.93	285.1 ± 4.33	High cellulose
9a	8 ♂	45.0 ± 2.26	190.6 ± 7.69	286.0 ± 8.94	High agar
10a	8 ♂	42.5 ± 1.98	200.5 ± 6.14	287.6 ± 5.97	No roughage
27	8 ♂	41.0 ± 2.12	195.4 ± 9.61	292.3 ± 8.09	No roughage

TABLE 5

*Cellulose diets for mice*

NUMBER	1-0	2-0	3-0	4-0
Dry liver	12	12	12	12
Casein	15	15	15	15
Lard	20	20	20	20
Starch	37	34	19	39
Yeast	5	5	5	5
Cod liver oil	5	5	5	5
Salt mixture	4	4	4	4
Cellulose	2	5	20	0

A year of preliminary studies feeding high roughage diets to white mice yielded consistent failures. These were due to inadequate supplements of B factors. Finally such supplements as vitavose were abandoned and yeast substituted. It was also found that cellulose must be ground very thoroughly to prevent selection of particles by white mice.

The diets shown in table 5 were used in the last series with mice.

Nine male mice were placed upon each of these diets shortly after weaning. The mean weights at the beginning of the experiment and again at the end of the tenth week are shown in table 6. Between the third and sixth weeks group 1-c and 4-c developed severe diarrhea and three of the animals in groups 1-c and 4-c died. Those upon the high cellulose diets seemed unaffected. These growth data for mice indicate they can grow quite normally upon diets containing large amounts of inert materials, such as cellulose. Other constituents in the diet must be sufficient for such growth, however.

*Cellulose and intestinal injury.* Although our earlier findings indicated rats can exist for about 600 days upon diets containing 20 per cent cellulose, there was the chance of internal injury of the gastro-intestinal tract until they slowly

TABLE 6  
*Weights of mice fed different roughage diets*

DIET NUMBER		1-c	2-c	3-c	4-c
Beginning		6.5	6.5	7.5	7.4
10th week		21.6	20.0	20.6	23.4

adapted themselves to such diets. As a preliminary experiment to detect such injury we fed twenty-one rats for the 4 months following weaning upon a diet of casein 25, salt mixture 4, lard 17, sugar 26, yeast 5, cod liver oil 3 and cellulose 20. As a control we fed seven rats of the same age upon the same diet with the cellulose replaced by cooked starch. At the end of the period the rats were killed. The stomach and intestines were removed. Some hemorrhage was found in the intestines of all animals that had been upon the high cellulose diets with a slight hemorrhage in three of the seven controls. Histological examination showed some increase in the connective tissue and some degeneration in the tubular epithelium in four of the high cellulose cases and one of the controls.

To obtain more information concerning this intestinal irritation, we divided twenty-four rats, ranging in weight from

70 to 100 gm., into four groups. They were fed the diets of table 7 for 33 days.

These diets were designed to have approximately the same crude fiber content. In nos. 3-D and 4-D an attempt was also made to equalize the protein. Rice hulls were selected because of their reputation for inflicting intestinal injury.

TABLE 7

*Diets used to study the intestinal irritation of rats by cellulose*

DIET NUMBER	1-D	2-D	3-D	4-D
Yeast	2	2	2	2
Casein	25	25	24	19
Starch	47	47	33	11
Lard	10	10	10	10
Cod liver oil	5	5	5	5
Bone meal	1	1	1	1
Sodium chloride	1	1	1	1
Calcium carbonate	1	1	1	1
Bran	—	—	—	50
Rice hulls	—	—	23	—
Cellulose (fine)	—	8	—	—
Cellulose (coarse)	8	—	—	—
Mean gain in weight (gm.)	118	142	48	33

TABLE 8

*Screen tests upon cellulose supplements*

	PER CENT RETAIN IN		PASSED
	20 mesh per inch	40 mesh per inch	
Cellulose (fine)	0.0	0.9	99.1
Cellulose (coarse)	4.6	53.3	42.1
Rice hulls	0.0	20.2	79.8
Bran	0.0	64.2	35.8

The crude fiber (diet 2-D) was ground as finely as our facilities permit.

The screen tests upon these cellulose products are given in table 8.

Lighter colored and softer feces were obtained from animals upon rice hulls and bran. The most growth resulted from the finely ground cellulose diet with very poor growth upon the natural sources of roughage.



At the conclusion of the experiment the intestinal tracts of the animals were examined carefully. One animal upon the finely ground cellulose showed a marked irritation in the duodenum. Irritation was found in three of the rats upon the coarsely ground material. Signs of irritation were found in all animals upon both the bran and rice hull diets. In about half these latter cases the irritation was pronounced. The bran used was an ordinary commercial product such as that used in animal feeds and not a purified product. Cellulose produces irritation in certain rats from a given group.

TABLE 9

*Composition of diets providing cellulose from various sources and the percentage of this cellulose digested by rats*

DIET NUMBER	1-E	2-E	3-E	4-E	5-E	6-E
Casein	25.0	27.0	26.0	28.0	30.0	30.0
Salt mixture	5.0	5.0	5.0	5.0	5.0	5.0
Lard	12.5	12.5	12.5	12.5	12.5	12.5
Sugar	20.0	29.5	15.0	26.5	30.0	34.0
Cod liver oil	5.0	5.0	5.0	5.0	5.0	5.0
Yeast	5.0	5.0	5.0	5.0	5.0	5.0
Starch	5.0	5.0	5.0	5.0	5.0	5.0
Bran	22.5	11.0	—	—	—	—
Beet pulp	—	—	26.5	13.0	—	—
Cellulose	—	—	—	—	6.0	3.0
Per cent crude fiber in diet	6.0	3.0	6.0	3.0	6.0	3.0
Per cent crude fiber digested	24.3 ± 1.1	31 ± 1.1	41.8 ± 3.4	42.7 ± 2.4	17.4 ± 1	20.8 ± 0.9

The fineness of grinding is a factor in this irritation since the more finely ground material produces less irritation. In experiments with men that have not been published, we have found a marked relation between the coarseness of the cellulose and irritation at the time of excretion. These experiments show little difference between the irritation from rice hulls and cellulose.

*The digestibility of cellulose from various sources and the fecal moisture.* In order to compare the digestibility of cellulose from various sources, we employed the diets of table 9.

The method used in this study was to place six rats in separate metabolism cages. After a preliminary period of 3 days they were fed a charcoal marker. They were then fed diet 1-E for 2 weeks and the feces collected for the same time after the marker appeared. After feeding the second marker, the animals were shifted to diet 2-E. After a 3-day preliminary period they were fed a third marker and the feces collected from diet 2-E for 2 weeks. The other diets were run in turn upon the same animals. The crude fiber of the feed and feces was then determined by the procedure of Kohmoto ('26). The digestibility of these various celluloses is shown at the bottom of table 9. Nearly twice as much of the beet pulp fiber is digested as that of the other products. Regenerated cellulose is the least digested. Bran is intermediate. In the case of both bran and regenerated cellulose the 3 per cent level is digested slightly better than the 6 per cent one. Beet pulp cellulose is the most widely digested by rats.

We appreciate that the crude fiber determination probably measures many other substances than cellulose. There are also a number of different forms of cellulose that we are unable to distinguish to-day. The fact that beet pulp crude fiber seems more readily digestible than other forms probably means there are substances present in beet pulp that are analyzed as crude fiber, but that are quite different in digestibility. The solution of such problems as the relative digestibility of cellulose in plant products of different ages, such as lettuce or carrots, awaits the day of better analytical methods.

*Cellulose and the weight of feces excreted.* In order to determine the influence of cellulose upon the weight of feces excreted, we placed six adult rats in separate metabolism cages. A diet consisting of dried egg albumin 10, starch 47, lard 20, sugar 20, mineral mixture 3 was fed. Vitamin supplements of 100 mg. of Harris concentrate and a small allowance of the non-saponifiable fraction of cod liver oil were fed each day separately. The feed consumed and the dry weight of

the feces were determined for 6 weekly periods. Ten per cent of the starch was then replaced in the diet by cellulose and another series of collections was made for 6 weeks. The feed consumed and dry feces excreted for the six individuals for 6 weeks are shown in table 10. It is of interest to note that the dry weight of the feces increases beyond the amount that can be accounted for by the addition of the cellulose less its digestible portion.

TABLE 10  
*The effect of ingested cellulose upon the weight of rat feces*

RAT NUMBER	FEED CONSUMED GRAMS PER RAT PER 6 WEEKS	FECES EXCRETED (DRY) GRAMS PER 6 WEEKS
Cellulose		
1	663.0	132.6
2	457.0	94.7
3	509.9	110.8
4	488.5	99.5
5	475.1	97.3
6	593.0	123.6
Mean	531.1 $\pm$ 22.1	109.7 $\pm$ 4.3
No cellulose		
1	442	41
2	479	41
3	427	38
4	309	25
5	508	47
6	482	42
Mean	441.2 $\pm$ 19.6	39.0 $\pm$ 2.0

During the 6 weeks of cellulose feeding the average rat ate 90 gm. of feed above that consumed without cellulose. But only 53 gm. of cellulose were ingested. If we assume that 17 per cent of this cellulose was digested, we would expect an increase of 44 gm. in the dry feces excreted. But the dry feces increased by an average of 70 gm. There may have been an average loss of 26 gm. of nutrients in addition to the cellulose loss in the feces in the course of 6 weeks.

We cannot say at this time whether this increase is due to the sweeping out of other nutrients by the cellulose or to increased cellular residues removed from the intestine. There

is certainly a marked difference in the fecal volume and weight with the ingestion of large amounts of cellulose.

*Fecal moisture in relation to cellulose in the diet.* Since fecal moisture is of real importance in the passage of feces, we ran a similar experiment to determine the moisture in feces when the cellulose of the diet originated from various sources. In figure 2, we have shown the moisture of these fresh feces. These determinations were made by placing the animal upon an experimental diet for 10 days. Five days after the beginning of the feeding, a 12-hour collection of the

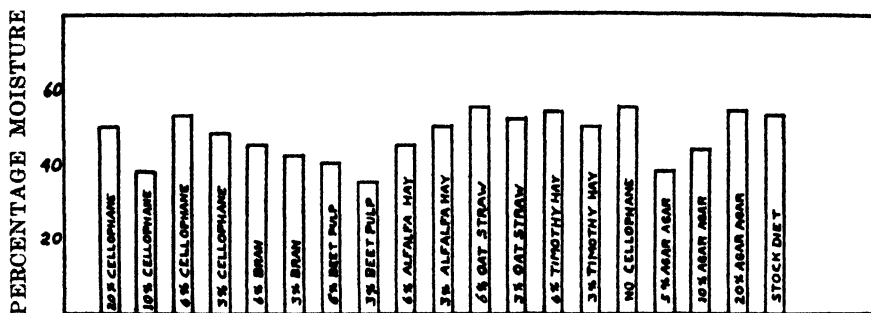


Fig. 2 Percentage moisture in the feces of rats in which the cellulose originated from various sources.

feces was made. They were placed in a tightly stoppered weighing bottle immediately after excretion. They were dried at 105° C. These data indicate that with the exception of agar the moisture per cent carried by the feces is not related to the content of inert material or its source. This is in conflict with the theory that the fecal moisture is governed by the cellulose. We realize that this cannot be a generalization, since we have studied only one species. Everyone appreciates the distinct properties of the feces of various species and that moisture is one of these characteristics.

#### SUMMARY

In preliminary experiments male rats fed 10 to 20 per cent cellulose diets were found to outlive the same sex fed a stock diet. This increase in the life span of these animals was

probably due to the slow rate at which they attained maturity rather than to a direct influence of the cellulose. However, it shows that the rat intestine is capable of tolerating these extreme levels of cellulose for a period in excess of that regarded as the normal life span. After a series of trials it proved possible to adjust the composition of the diet so that as good growth was obtained with diets containing 20 per cent cellulose or agar as with diets lacking roughage. Mice can also tolerate such levels of roughage and grow normally. In the course of an epidemic of diarrhea among the mice, it was observed that the animals on the high cellulose diet were not affected. In some rats signs of intestinal irritation were found. These seem to be characteristic of individuals. The more finely ground cellulose produced the least irritation. Rice hulls were no more irritating than bran or regenerated cellulose. No evidence was found that this irritation leads to permanent injury. In studies to determine the relative digestibility of crude fiber from beet pulp, bran and regenerated cellulose, the beet pulp was found to be digested to the extent of 40 per cent, while the other two were about half as well digested. In the case of bran and regenerated cellulose 3 per cent levels are slightly better digested than 6 per cent ones. In a determination of the effect of the cellulose ingested upon the feces excreted it was found that the dry weight of these feces increases beyond an amount that can be ascribed to the ingested cellulose. Either there is a decreased digestibility of nutrients or the cellulose causes increased losses in the feces of such materials as epithelial cells from the intestine. No relation could be established between the moisture content of the feces and the level of roughage ingested, except in the case of agar. In the case of this material there was a direct relationship.

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## LITERATURE CITED

- CAMPBELL, H. LOUISE 1928 Reproduction and longevity of experimental animals as research criteria in the chemistry of nutrition. Dissertation, Columbia University.
- KOHMOTO, T., AND S. SAKAGUCHI 1926 Über die Zellulosebestimmung der menschlichen Fazes und die verdauung der Nahrungszellulose. J. Biochem. (Japan), vol. 6, p. 61.
- MCCAY, C. M. 1929 The effect of roughage upon growth. Proc. Soc. Exp. Biol. and Med., vol. 27, p. 209.
- 1933 Is longevity compatible with optimum growth? Science, vol. 77, p. 410.
- MAYNARD, L. A. 1930 A diet for stock rats. Science, vol. 71, p. 192.
- ROSE, MARY S., GRACE MACLEOD, ELLA McC. VAHLTEICH, ESTER H. FUNNEL, AND CATHARINE L. NEWTON 1932 The influence of bran on the alimentary tract. J. Am. Diet. Assoc., vol. 8, p. 133.
- SLONAKER, J. R. 1912 The effect of a strictly vegetable diet on the spontaneous activity, the rate of growth and the longevity of the albino rat. Leland Stanford Univ. Pub.



# A COMPARISON OF VITAMINS B AND G IN CANNED STRAINED FOODS <sup>1</sup>

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The recognition of several factors in the undifferentiated vitamin B required for rat growth has necessitated repetition of earlier assays. Since the antineuritic vitamin B is susceptible to destruction by moist heat, we might expect canned foods to vary in vitamin B content and its ratio to vitamin G potency, depending upon the heat process used in the canning method. Tests performed on canned strained foods give a measure of their value as sources of vitamins in the form in which they are widely used for infant and convalescent diets. Therefore, we have determined the vitamin B and G potency and thus the ratio of these two factors in some canned strained foods: tomatoes, carrots, peas, green beans, beets, spinach, a vegetable soup, and a canned cereal.

## EXPERIMENTAL

The determinations of the vitamin B and G content were made upon rats raised in this laboratory, 28 to 29 days old and weighing 35 to 60 gm. They were kept in individual cages with raised screen bottoms, 3 meshes to the inch. In order to control coprophagy more closely, we adopted the use of rat 'harnesses' as described by Page ('32) for the tests of both vitamins. In other respects, the usual technic for conducting an animal test was observed as described in a previous paper (Hanning, '33).

<sup>1</sup> Financed by a grant from the Gerber Products Company.



The vitamin B deficient diet was that of Chase and Sherman ('31), except that the casein was extracted with dilute acetic acid, and the vitamin G deficient diet that of Bourquin and Sherman ('31). While these diets may not be entirely adequate in regard to other B vitamins required by the rat, it was felt that with the short test period of 5 weeks and the natural foodstuffs being tested, there probably was no significant deficiency of any except the one vitamin being determined.

After a preliminary depletion period averaging 16.4 days on the B deficient diet and 13.8 days on the G deficient diet, the supplemental feedings were begun and continued 5 weeks. Except for the rats fed the B deficient diet plus canned strained spinach and to some extent, beets, there was no great difficulty in obtaining complete consumption of the supplements. The data of all rats not consuming the required amount were discarded.

The supplements used throughout were furnished us by the Gerber Products Company, the canning method being briefly described in a previous paper ('33). The tomatoes, carrots, green beans and beets were representative samples of the entire 1932 season pack. Spinach no. 3 was a similarly representative sample of spring spinach of 1932, while spinach no. 1 was from 1 day's pack of fall spinach canned in 1931. Peas no. 1 was of a variety known as Perfection and no. 2 Thomas Laxton, both canned in 1931 from 1 day's pack. The vegetable soup and the canned cereal were taken from the warehouse as representing the usual product of 1932.

## RESULTS

### *I. Vitamin B content of the vegetables*

The data of vitamin B tests of the vegetables are presented in table 1. The potencies are expressed in terms of units as that amount which when fed daily produced an average gain of 3 gm. per week. The data indicate that 2 gm. of canned strained tomatoes or 3 gm. of strained peas, either no. 1 or no. 2, with somewhat different total solids content, gave more than unit growth.

On the other hand, it required 7 gm. of canned strained carrots for 1 unit, but 10 gm. of strained green beans or beets contained more than 1 unit of vitamin B. Because of the difficulty of obtaining complete consumption, the largest amount of spinach fed was 6 gm. Receiving this quantity, the rats lost in weight to approximately the same extent as those fed 5 gm. of strained green beans, which amount contained

TABLE 1

*Record of rats fed canned strained vegetables as sources of vitamin B*

AMOUNT OF CANNED STRAINED FOOD FED DAILY <sup>1</sup>	NUMBER OF RATS	WEIGHT AT DEPLETION <sup>2</sup>	DAILY FOOD CONSUMPTION <sup>2</sup>	AVERAGE WEEKLY GAIN <sup>2</sup>	UNITS OF VITAMIN B PER OUNCE
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
1 gm. tomatoes	4	66.75	4.04	+ 0.30	24.0
2 gm. tomatoes	6	68.67	5.34	+ 4.75	
3 gm. peas no. 1	8	64.75	4.01	+ 3.55	
4 gm. peas no. 1	7	63.71	4.56	+ 4.94	7.0-8.0
3 gm. peas no. 2	8	65.00	4.14	+ 3.80	
5 gm. carrots	5	63.20	4.09	+ 1.44	
7 gm. carrots	6	68.67	4.83	+ 3.03	4.3
10 gm. carrots	6	67.16	5.29	+ 6.80	
4 gm. green beans	6	59.83	4.99	— 4.03	
5 gm. green beans	8	58.63	4.05	— 0.73	4.7
10 gm. green beans	7	68.00	5.22	+ 4.69	
7 gm. beets	4	69.75	3.96	— 1.20	
10 gm. beets	5	68.20	4.18	+ 3.44	3.3
4 gm. spinach no. 3	8	63.63	4.26	— 4.75	2.5
6 gm. spinach no. 3	4	64.60	4.34	— 1.32	
None	33	64.76	2.95 <sup>3</sup>	— 6.93 <sup>3</sup>	

<sup>1</sup> Of the representative samples, 1 gm. of canned strained tomatoes was produced from 2.65 gm. raw; of strained carrots, from 0.65 gm. raw; of strained green beans, 0.64 gm. raw; of strained beets, from 0.85 gm. raw; and 1 gm. canned strained spinach (no. 3) was produced from 1 gm. raw spinach. Although the peas were not representative of the entire season's pack, 1 gm. of the strained product was approximately equivalent to 0.73 gm. raw peas.

<sup>2</sup> Averages for the group.

<sup>3</sup> All except three of the rats died before the end of the test—the average period being 22.8 days. Averages are for the survival instead of the entire test period.

$\frac{1}{2}$  unit of vitamin B. Therefore, 12 gm. of strained spinach probably contained 1 unit. These potencies are expressed also in terms of units per ounce in the last column of table 1.

## II. Vitamin G content of the vegetables

The record of the vitamin G tests of the vegetables is presented in table 2. Here, again, the unit is defined as the

TABLE 2  
*Record of rats fed canned strained vegetables as sources of vitamin G*

AMOUNT OF CANNED STRAINED FOOD FED DAILY <sup>1</sup>	NUMBER OF RATS	WEIGHT AT DEPLETION <sup>2</sup>	DAILY FOOD CONSUMPTION <sup>2</sup>	AVERAGE WEEKLY GAIN <sup>2</sup>	UNITS OF VITAMIN G PER OUNCE
		gm.	gm.	gm.	
3 gm. spinach no. 1	8	53.00	5.40	+ 4.05	7.5-11.8
1 gm. spinach no. 3	7	50.70	4.38	— 0.40	
3 gm. spinach no. 3	8	51.38	4.92	+ 2.28	
5 gm. spinach no. 3	7	50.38	6.07	+ 4.80	
3 gm. peas no. 1	7	45.43	4.21	+ 2.69	7.5- 8.6
5 gm. peas no. 1	14	51.72	4.91	+ 5.50	
7 gm. peas no. 1	6	45.17	4.60	+ 6.20	
5 gm. peas no. 2	12	52.67	5.20	+ 4.37	
4 gm. tomatoes	7	49.85	5.10	+ 2.43	4.0- 5.0
5 gm. tomatoes	5	52.60	5.06	+ 3.72	
3 gm. green beans	6	55.00	5.23	+ 1.07	6.0
5 gm. green beans	7	52.57	5.02	+ 2.94	
7 gm. beets	7	50.67	4.49	+ 2.23	3.3
10 gm. beets	5	49.00	4.81	+ 3.56	
7 gm. carrots	8	49.75	3.90	+ 0.95	2.5
10 gm. carrots	7	47.67	4.04	+ 2.43	
None	28 <sup>3</sup>	51.86	4.11	— 0.91	

<sup>1</sup> Of the representative samples, 1 gm. of canned strained tomatoes was produced from 2.65 gm. raw; of strained green peas, 0.64 gm. raw; of strained beets from 0.85 gm. raw; of strained carrots, 0.65 gm. raw; and 1 gm. canned strained spinach no. 3 was produced from 1 gm. raw spinach. Spinach no. 1 was not a representative sample. Although the peas were not representative of the entire season's pack, 1 gm. of the strained product was approximately equivalent to 0.73 gm. raw peas.

<sup>2</sup> Average for the group.

<sup>3</sup> One rat died 3 days before the end of the test period; the others survived the entire period.

amount which when fed daily produced a 3-gm. gain per week. The strained peas and spinach were very good sources of vitamin G but the two samples of each were different in potency. Of spinach no. 1 containing 6.78 per cent total solids, 3 gm. gave more than unit growth; of no. 3 with 6.30 per cent total solids, less. Peas no. 1 gave better growth than no. 2. This can probably be explained by the higher total solid content, 12.33 per cent in no. 1, while no. 2 contained 10.83 per cent total solids. Thus, 3 to 4 gm. of canned strained spinach or peas contain about 1 unit of vitamin G. Approximately unit growth was obtained upon 4 to 5 gm. strained tomatoes, 5 gm. green beans, or 10 gm. of strained beets. Of strained carrots, 10 gm. gave less than unit growth. The values calculated to units per ounce are also listed.

### *III. Tests of cereal and vegetable soup*

In comparison with the data on vegetables, our tests of the canned strained cereal indicate that 4 gm. contain 1 unit vitamin B and 2.5 gm., 1 unit vitamin G. The large quantity of milk used in its manufacture is responsible for the high vitamin G potency. The vegetable soup contained equal amounts of the B and G vitamins, 10 gm. being required for 1 unit of each.

### DISCUSSION

It is interesting to note that the canned strained tomatoes contain, according to our tests, about three times as many units of vitamin B as of vitamin G. This is indicated by comparing the values as expressed in units per ounce. The strained carrots and peas contain about one and a half times as many units of vitamin B as vitamin G, while beets have equal amounts of each vitamin. On the other hand, the canned strained green beans contain twice and spinach about three to four times as many units of vitamin G as vitamin B. Thus, in the canned strained tomatoes, carrots, and peas, vitamin B predominates; in spinach and green beans, vitamin G, but equal amounts of each vitamin are found in beets.

An exhaustive study of differentiated vitamin B determinations on rats has been made by Roscoe in England ('30, '31 a, '31 b) who expressed the potency in terms of that of dried brewer's yeast. The amount of yeast (0.05 gm. for B and 0.1 gm. for G) which would induce 50 to 60 gm. gain in 5 weeks was taken as 100. On that basis she rated spinach and carrots as 5 to 10 and tomatoes as 2.5 to 5 in vitamin B; in vitamin G, spinach as 10 to 20, carrots 5 to 10 and tomatoes 2.5 to 5. Since it requires twice as much yeast for her standard of potency of 100 in vitamin G as in vitamin B, spinach would have about four times as much vitamin G as B, if rated on growth as in our own data. In the same manner, carrots and tomatoes would have twice as much vitamin G as vitamin B. In our work upon canned strained foods, as noted above, we found three to four times as much vitamin G as vitamin B in spinach, but three times as much vitamin B as vitamin G in tomatoes, and one and a half times more vitamin B than vitamin G in carrots. That is, in the two studies, the ratio is the same for spinach, but our samples of carrots and tomatoes contained a much greater proportion of vitamin B to vitamin G.

In this country, the work of Rogers ('28) indicates a greater concentration of vitamin G than vitamin B in spinach and green beans whether raw or cooked or canned. Jones and Nelson ('30) fed juice from canned vine-ripened tomatoes. From the growth curves presented, it would appear that 5 cc. of the juice contained much more than 1 unit vitamin B in terms of the 3 gm. gain per week standard. Hartley is quoted by Sherman ('31) as having determined the vitamin G in the juice from canned tomatoes to be 0.16 units per gram. Day ('31) studied the vitamin G content of a number of vegetables feeding the raw food. He lists carrots and beets as containing 0.5 unit and winter spinach 1 unit per gram, based on an 8 weeks' test.

We realize, however, that it is difficult to make direct comparisons as to the vitamin B and G potency of the various foods because of varying factors in the experimental method.

This is especially true of the control of coprophagy by 'rat harnesses.' We have found in this laboratory that their use results in much lower but more reliable and concordant data for both vitamins B and G. How far these tests are influenced by a lack of vitamin B<sub>4</sub> or other B factors cannot be surmised.

#### SUMMARY

From data collected under very carefully controlled conditions with the use of rat 'harnesses' to prevent coprophagy, it was found that 1 ounce of canned strained tomatoes contained from 20 to 24 units vitamin B and 6.7 units vitamin G; of canned strained peas, 7 to 8 units vitamin B and 7.5 to 8.6 units vitamin G; of canned strained carrots 4.3 units vitamin B and 2.5 units vitamin G. The canned strained beets contained 3.3 units of each vitamin, and the canned strained green beans contained 4.7 units vitamins B, and 6 units vitamin G. The canned strained spinach was low in vitamin B, containing probably 2.5 units, with 7.5 to 11.8 units of vitamin G per ounce.

In three of the canned strained vegetables: tomatoes, peas and carrots, there was more vitamin B than vitamin G; in green beans and spinach, more vitamin G than vitamin B; and in canned strained beets, equal amounts of each vitamin.

A canned strained cereal contained 7.5 units vitamin B, and 12 units vitamin G per ounce. In a canned vegetable soup there were 3 units of each vitamin per ounce.

## LITERATURE CITED

- BOURQUIN, A., AND H. C. SHERMAN 1931 A quantitative determination of vitamin G (B<sub>2</sub>). J. Am. Chem. Soc., vol. 53, p. 3501.
- CHASE, E. F., AND H. C. SHERMAN 1931 A quantitative study of the determination of the antineuritic vitamin B. J. Am. Chem. Soc., vol. 53, p. 3506.
- DAY, P. A. 1931 The vitamin G content of leaf and root vegetables. S. Med. J., vol. 24, p. 876.
- HANNING, F. 1933 Canned strained vegetables as sources of vitamin A. J. Am. Diet. Assoc., vol. 9, p. 295.
- JONES, D. B., AND E. M. NELSON 1930 Vitamin content of ethylene-treated and untreated tomatoes. Am. J. Pub. Health, vol. 20, p. 387.
- PAGE, J. W. 1932 An improvement in experimental method for investigation of vitamin G. Proc. Exp. Biol. Med., vol. 30, p. 67.
- ROGERS, E. C. 1928 Quantitative determination of vitamin B in spinach, and string beans, with a preliminary report on the vitamin A content of the same foods. Dissertation, Univ. of Missouri.
- ROSCOE, M. H. 1930 The distribution of the vitamin B complex. I. Leafy vegetables. Biochem. J., vol. 24, p. 1754.
- 1931 a The distribution of the vitamin B complex. II. Root vegetables. Biochem. J., vol. 25, p. 1205.
- 1931 b The distribution of the vitamin B complex. III. Fruits. Biochem. J., vol. 25, p. 2050.
- SHERMAN, H. C., AND S. L. SMITH 1931 The vitamins. The Chemical Catalog Co., Inc., New York. 2nd ed., p. 139.

# INANITION AS A FACTOR IN VITAMIN G DEFICIENCY

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Since the experiments of Smith and Hendrick ('26) and Goldberger and Lillie ('26) it has been known that animals restricted to a vitamin G deficient diet become stunted and frequently develop a characteristic dermatitis. The skin lesions are undoubtedly a manifestation of the specific lack of vitamin G. The failure to grow and the accompanying changes in the tissues and organs of the body have been tacitly attributed to the vitamin deficiency also. Little attention has been directed to the food intake of the animals. While the food consumption in vitamin G deficiency has been recognized as subnormal, yet, in comparison with the anorexia of vitamin B deficiency, it is sufficiently high to have led to the conclusion that vitamin G plays a negligible part in the maintenance of the appetite. It remained for Graham and Griffith ('33) to emphasize the importance of vitamin G as an extrinsic factor in the physiological mechanism controlling the consumption of food. The present experiments were planned to study the effects of vitamin G deficiency, as opposed to simple caloric insufficiency, on some of the tissues of the body. The mouse was selected as the experimental animal, following the work of Bing and Mendel ('29).

## EXPERIMENTS

Three-week-old mice were removed from their mothers and divided into three groups. All animals were fed a basal diet



plus 2 drops of cod liver oil daily. The 'normal' animals received 250 mg. of dried yeast per day and the mice in the 'G-deficient' group received 0.1 cc. of a vitamin B containing sirup. The 'controls' received the same amount of yeast as did normal animals, but the total food intake was restricted to the amount consumed by the G-deficient mice.

The basal diet was prepared by mixing 200 gm. of extracted casein, 265 gm. of cornstarch, 200 gm. of lard and 40 gm. of the McCollum salt mixture. The vitamin B preparation was made by extracting 2 kg. of wheat germ (which contained 5.6 per cent moisture) with 5 liters of ordinary alcohol. The filtrate from the extracted wheat germ was evaporated in vacuo at a temperature not exceeding 60° until all the alcohol was removed. The residue was diluted with two volumes of water, placed in the refrigerator overnight, and then filtered while cold. The product consisted of a sirupy solution which contained 25 per cent solids and 0.2 per cent ash. Assay of the solution showed that 0.1 cc. provided sufficient vitamin B to meet the growth requirements of mice.

We have found that animals fed upon the G-deficient diet fail to grow and die in an average time of 7 weeks. About 75 per cent of the animals develop an ulcerative dermatitis, usually in the axillary region or beneath the lower jaw. No signs of changes in the eyeball have been observed macroscopically. The addition of small amounts of crystalline carotene, from 0.001 mg. to 0.100 mg. per day has no influence on the survival time or on the incidence of dermatitis.

In the present study the experimental period was limited to 6 weeks so as to avoid the factor of complete anorexia which characterizes the terminal stages of vitamin G deficiency. At the end of this time, the mice were sacrificed and their tissues were examined chemically. The hemoglobin concentration of the blood and the number of red cells were determined by the method of Heinle and Bing ('33). The serum protein concentration was estimated by digestion of 0.025 cc. of blood serum with sulfuric acid and 30 per cent  $H_2O_2$ , followed by direct Nesslerization essentially as described by Myers ('24).

The femurs were removed and analyzed by the methods employed by Karelitz and Shohl ('27). The fat was extracted by the Bloor method ('26) from the entire carcasses of mice after the removal of the stomach and intestines. This procedure yields a solution of the lipids in petroleum ether. The total fat was estimated by evaporating the solvent and weighing the residue. Phospholipids were determined by analysis of an aliquot portion of the petroleum ether solution for phosphorus by the method of Kuttner and Lichtenstein ('32).

#### RESULTS AND CONCLUSIONS

The average values of the figures obtained on individual mice are presented in tables 1 and 2.

*Growth and food consumption.* The calorie-deficient and G-deficient animals were stunted in weight. The total average food intakes of the stunted mice were approximately 60 per cent of normal.

Since the early work of Karr ('20) and Cowgill ('21) the vitamin B complex has been recognized as a dietary factor that exerts a specific effect on the appetite. Burack and Cowgill ('31) showed that dogs deprived of the vitamin B complex, until they no longer consumed their food, had their appetites restored by administration of vitamin B but not by vitamin G. Our data show that vitamin B evokes a maintenance appetite for a limited time, probably until the animal's available store of vitamin G is depleted, when the appetite fails.

*Composition of the blood.* The average values of the hemoglobin concentration and the number of red cells were slightly below normal in both G-deficient and calorie-deficient mice, the cells being less affected than the hemoglobin. Sure, Kik and Smith ('31) and Guha and Mapson ('31) have reported a somewhat more marked decrease in the hemoglobin and red blood cells of rats maintained on a G-deficient diet. Normal mice at the age of 3 weeks have about 8.0 gm. of hemoglobin per 100 cc. of blood, which is less than the hemoglobin concentration of the stunted animals.

The serum protein concentration in the calorie restricted mice was less than that of the G-deficient animals, but, because of the small number of observations, further experiments on this point would be desirable.

*Composition of the femurs.* The figures in table 1 show that the stunted mice had lighter bones with less ash than normal animals. In 3-week-old mice weighing 9.5 gm., the femur weighs 10.5 mg. when dried, the ash content averages 4.5 mg., and the percentage of ash in the dried and defatted bone is 44.3 per cent. It is therefore evident that in stunted

TABLE 1  
*Average composition of blood and bones in vitamin G deficiency*

	NORMAL	CONTROL	G-DEFICIENT
Number of mice	7	7	7
Initial weight	10.8 gm.	10.4 gm.	10.0 gm.
Final weight	21.9 gm.	9.8 gm.	9.7 gm.
Food intake	98.1 gm.	54.6 gm.	55.7 gm.
Hemoglobin	12.77 gm.	10.27 gm.	11.18 gm.
Red blood cells	9.83 million	9.06 million	9.60 million
Serum proteins	5.36 gm.	3.95 gm.	5.04 gm.
Weight dried femur	33.2 mg.	20.3 mg.	19.7 mg.
Extracted weight	31.6 mg.	19.7 mg.	18.4 mg.
Ash weight	18.8 mg.	10.1 mg.	9.5 mg.
Per cent ash	59.3 per cent	50.9 per cent	51.8 per cent

growth, produced by either calorie or vitamin G restriction, the long bones continue to grow at a sub-normal rate. The dried weight of the femurs is almost doubled and the weight of the ash is more than doubled in 6 weeks, even though the body weight remains practically constant. The phenomenon of persistent skeletal growth in stunted rats has been demonstrated by Winters, Smith and Mendel ('27).

*Body fat and total phospholipid content.* The data presented in table 2 show a very close resemblance in the amount of fat and phospholipid in the two groups of stunted mice. For comparison, five normal mice were sacrificed at the age of 3 weeks and analyzed. The average values were as follows:

live weight, 9.5 gm.; net body weight, 8.9 gm.; ether extract, 1.13 gm.; lipid phosphorus, 5.34 mg. These figures indicate that the fat content is not only very low in the stunted mice, but it is actually much less than the animals presumably have at the beginning of the experiment. It is of interest that the phospholipid content of the body remains relatively constant in mice that are stunted, even though the neutral fat practically disappears. This may be considered as further evidence of the importance of phospholipids as essential components of the tissues, in accordance with the conception of the *élémente constant* of Terroine and Belin ('27).

TABLE 2

*Average content of body fat and phospholipids in vitamin G deficiency*

	NORMAL	CONTROL	G-DEFICIENT
Number of mice	6	7	7
Initial weight	9.3 gm.	9.7 gm.	9.3 gm.
Final weight	20.9 gm.	10.2 gm.	9.7 gm.
Food intake	94.9 gm.	63.9 gm.	63.5 gm.
Net body weight	18.0 gm.	8.8 gm.	7.8 gm.
Ether extract	2.86 gm.	0.48 gm.	0.46 gm.
Lipid P	7.94 mg.	5.79 mg.	5.78 mg.

## SUMMARY

Young mice, receiving a vitamin G deficient diet for 6 weeks, consume approximately 60 per cent of the caloric intake of other mice fed a complete diet. In both calorie-deficient and G-deficient mice the body weight remains approximately constant, the neutral fat almost disappears from the body, the total phospholipid content remains unchanged, and the bones continue to grow at a sub-normal rate. The number of red blood cells and the concentration of hemoglobin and of serum protein are slightly lower in stunted mice than in normal animals of the same age. Inanition is thus a significant feature of the syndrome of vitamin G deficiency. Both vitamins G and B are required for the maintenance of the normal appetite.

## LITERATURE CITED

- BING, F. C., AND L. B. MENDEL 1929-1930 The vitamin B and the vitamin G requirements of the albino mouse. *J. Nutrition*, vol. 2, p. 49.
- BLOOR, W. R. 1926 Distribution of unsaturated fatty acids in tissues. I. Beef heart muscle. *J. Biol. Chem.*, vol. 68, p. 33.
- BURACK, E., AND G. R. COWGILL 1931 Anorexia characteristic of lack of the vitamin B complex: The roles of the individual components. *Proc. Soc. Exp. Biol. and Med.*, vol. 28, p. 750.
- COWGILL, G. R. 1921 A contribution to the study of the relation between vitamin B and the nutrition of the dog. *Am. J. Physiol.*, vol. 57, p. 420.
- GOLDBERGER, J., AND R. D. LILLIE 1926 A note on an experimental pellagra-like condition in the albino rat. *Pub. Health Rep.*, vol. 41, p. 1025.
- GRAHAM, C. E., AND W. H. GRIFFITH 1933 Studies on growth. II. The effect of vitamins B and G on the consumption and utilization of food. *J. Nutrition*, vol. 6, p. 195.
- GUHA, B. C., AND L. W. MAPSON 1931 The role of certain dietary factors in the formation of erythrocytes. *Biochem. J.*, vol. 25, p. 1674.
- HEINLE, R. W., AND F. C. BING 1933 Studies in the nutritional anemia of the rat. VIII. A method for the estimation of hemoglobin and erythrocytes on a single small sample of blood. *J. Biol. Chem.*, vol. 101, p. 369.
- KARELITZ, S., AND A. T. SHOHL 1927 Rickets in rats. I. Metabolism studies on high calcium-low phosphorus diets. *J. Biol. Chem.*, vol. 73, p. 655.
- KARR, W. G. 1920 Some effects of water-soluble vitamins upon nutrition. *J. Biol. Chem.*, vol. 44, p. 255.
- KUTTNER, T., AND L. LICHTENSTEIN 1932 Micro colorimetric studies. III. Estimation of organically bound phosphorus. A system of analysis of phosphorus compounds in blood. *J. Biol. Chem.*, vol. 95, p. 661.
- MYERS, V. C. 1924 Practical chemical analysis of blood. 2nd revised ed. C. V. Mosby Co., St. Louis.
- SHEEMAN, H. C., AND M. R. SANDELS 1930-1931 Further experimental differentiation of vitamins B and G. *J. Nutrition*, vol. 3, p. 395.
- SMITH, M. I., AND E. G. HENDRICK 1926 Some nutrition experiments with brewer's yeast with especial reference to its value in supplementing certain deficiencies in experimental rations. *Pub. Health Rep.*, vol. 41, p. 201.
- SURE, B., M. C. KIK AND M. E. SMITH 1931 Hematopoietic function in avitaminosis. VI. Vitamin G deficiency. *Proc. Soc. Exp. Biol. and Med.*, vol. 28, p. 498.
- TERROINE, E. F., AND P. BELIN 1927 L'élément constant des lipides: ses caractères. *Bull. Soc. Chim. biol.*, T. 9, p. 12.
- WINTERS, J. C., A. H. SMITH AND L. B. MENDEL 1927 The effects of dietary deficiencies on the growth of certain body systems and organs. *Am. J. Physiol.*, vol. 80, p. 576.

# CAROTENE AND VITAMIN A REQUIREMENTS FOR WHITE LEGHORN CHICKS

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FOUR TEXT FIGURES AND ONE PLATE

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The object of this study was to obtain data regarding the minimum requirements of carotene and vitamin A for White Leghorn chicks, and to determine whether such data might have an application in a quantitative test for vitamin A.

The battery brooder used was the conventional type with electric heating units and thermostatic control. For each compartment containing a heating unit there was a duplicate compartment without a heating unit. A removable partition between the two compartments made it possible to provide additional space for each group as required. Food and water troughs were provided in the usual manner. The brooder was located in a well-insulated room ventilated only by windows and protected from direct sunlight. Before using the brooder, it was taken apart, scrubbed with soap and water and steamed.

## EXPERIMENTAL

In order to obtain the desired information, we divided 612 day-old White Leghorn chicks into the following groups:

Group A, composed of ten groups of ten chicks each. The chicks in these groups received graduated doses of carotene in oil of known potency (sample A) for 30 days in addition to the basal diet which they received throughout the test period.

The doses used were decimal graduations from 10 to 100 A.D.M.A. units.

Group B, composed of ten groups of ten chicks each. The chicks in these groups received graduated doses of carotene in oil of unknown potency (sample B) for 30 days in addition to the basal diet which they received throughout the test period. The doses used were decimal graduations from 10 to 100 A.D.M.A. units.

Group C, composed of nine groups of ten chicks each. The chicks in these groups received graduated doses of carotene in oil of unknown potency (sample C) for 30 days in addition to the basal diet which they received throughout the test period. The doses used were decimal graduations from 10 to 90 A.D.M.A. units.

Group D, composed of nine groups of ten chicks each. The chicks in these groups received graduated doses of cod liver oil of known potency (sample D) for 30 days in addition to the basal diet which they received throughout the test period. The doses used were decimal graduations from 10 to 90 A.D.M.A. units.

The control group contained 232 chicks, which received the basal diet only.

Beginning with the first day, all groups received the vitamin A-free basal diet, which consisted of:

- 52½ pounds white corn, ground
  - 10 pounds wheat bran
  - 15 pounds wheat middlings
  - 10 pounds meat scrap
  - 10 pounds skimmed milk powder
  - 2 pounds calcium carbonate
  - ½ pound sodium chloride
- 100 A.D.M.A. units of vitamin D per chick per day.

The carotene and vitamin A solutions were fed daily by mouth with standardized medicine droppers for 30 days starting with June 17, 1933. These solutions were diluted with refined cottonseed oil so that one drop carried the particular graduated dose of carotene or vitamin A plus 100 A.D.M.A. units of vitamin D. The control group received no carotene or vitamin A, but was given 100 units of vitamin D in one drop of the same oil used for diluting. All chicks received 100 Sherman units of vitamin D per day throughout the test period. The source of vitamin D was a vitamin D concentrate

from cod liver oil produced by the Zucker process (Columbia University). The relatively small amount of vitamin A normally present in this concentrate was destroyed by oxidizing at 100°C. with a current of air for 24 hours.

Inasmuch as the control group received no carotene or vitamin A throughout the experimental period and whereas the members of groups A, B, C, and D received various amounts of carotene or vitamin A during a period of 30 days, the control group became depleted in carotene and vitamin A much sooner than the other groups. If we assume that there exists a certain minimum number of units of vitamin A or carotene, X, which if supplied daily to each chick will permit it to live a normal life, then it is evident that the carotene or vitamin A supplied to the members of groups A, B, C, and D should permit them to live a certain number of days longer than the members of the control group. The average number of days of additional life and the number of units of carotene or vitamin A required by the animals of groups A, B, C, and D, respectively, to live an extra day over the average number of days lived by the animals of the control group was calculated and is presented with a summary of the experimental results in table 1.

The 232 controls which received no carotene or vitamin A supplement lived a grand total of 6310 days; an average of 27.2 days per chick.

In group A, in which carotene in oil of known potency was given, the average of ninety chicks showed that the group lived 1 additional day for each 114 A.D.M.A. units of vitamin A received. Since there were only ninety chicks in the C and D groups, the averages of the corresponding ninety chicks in the A and B groups were used. If, however, the total of 100 chicks was used, the number of A.D.M.A. units required to live 1 extra day was 116, as compared to the 114 with the ninety chicks.

Group B was given carotene in oil of unknown potency, but it was fed on the basis of having the same potency as the A group. On this basis, it took 160 A.D.M.A. units per chick



TABLE 1  
Summary of results. Carotene and vitamin A requirement experiments with 618 day-old White Leghorn chicks

	NUMBER OF VITAMIN A UNITS EACH CHICK RECEIVED DAILY										70	80	90	100	AVERAGE OF 100 CHICKS	AVERAGE OF 90 CHICKS
Group A, carotene in oil known potency (sample A)	Chicks in group	9	10	10	10	10	10	10	10	10	9	10	10	10	100	98
	Units group received	2630	5380	8460	10000	13700	17280	18900	18900	18900	17280	21920	24030	30000	152300	123360
	Days group lived	302	341	407	360	408	407	398	432	403	407	422	403	517	3985	3468
	Units per chick	292	538	846	1000	1370	1728	2100	2192	2403	1728	2192	2403	3000	1554	1390
	Average life per chick	33.6	34.1	40.7	36.0	40.8	40.7	44.2	44.2	40.3	40.7	44.2	40.3	51.7	40.6	39.4
	Extra days lived per chick	6.4	6.9	13.5	8.8	13.6	13.5	17.0	17.0	13.1	13.5	17.0	13.1	24.5	13.4	12.2
	Units required to live extra day	46	78	63	114	101	128	124	129	183	101	129	183	122	116	114
Group B, carotene in oil unknown potency (sample B)	Chicks in group	10	10	10	10	10	10	10	10	10	10	10	10	10	100	100
	Units group received	2550	5960	8010	10760	13800	17040	18690	20560	23850	17040	20560	23850	27000	148220	121220
	Days group lived	295	382	308	360	372	401	378	355	356	401	378	355	355	3560	3205
	Units per chick	255	596	801	1076	1380	1704	1869	2056	2385	1704	2056	2385	2700	1482	1347
	Average life per chick	29.5	38.2	30.8	36.0	37.2	40.1	37.6	35.5	35.6	40.1	37.6	35.5	35.5	35.6	35.6
	Extra days lived per chick	2.3	11.0	3.6	8.8	10.0	12.9	10.4	8.3	8.4	10.0	10.4	8.3	8.3	8.4	8.4
	Units required to live extra day	111	54	223	122	138	132	180	248	284	132	180	248	325	176	160
Group C, carotene in oil unknown potency (sample C)	Chicks in group	10	10	9	10	10	10	10	10	10	10	10	10	10	100	89
	Units group received	2790	4840	7050	10960	13950	15900	21000	23280	25290	13950	21000	23280	25290	125060	125060
	Days group lived	326	272	289	344	325	342	413	386	386	342	413	386	386	386	3083
	Units per chick	279	484	783	1096	1395	1590	2100	2328	2529	1395	2100	2328	2529	1405	1405
	Average life per chick	32.6	27.2	32.1	34.4	32.5	34.2	41.3	38.6	38.6	34.2	41.3	38.6	38.6	34.6	34.6
	Extra days lived per chick	5.4	0.0	4.9	7.2	6.3	7.0	14.1	11.4	11.4	6.3	14.1	11.4	11.4	7.4	7.4
	Units required to live extra day	52	.....	160	152	263	227	149	204	222	263	149	204	222	190	190
Group D, cod liver oil (sample D)	Chicks in group	10	9	10	10	10	10	10	10	10	10	10	10	10	89	89
	Units group received	2690	5220	9000	11360	14900	16740	20370	20370	24840	16740	20370	20370	24840	125440	125440
	Days group lived	311	328	394	380	409	377	422	422	416	377	422	416	416	3397	3397
	Units per chick	269	520	900	1136	1490	1674	2037	2037	2484	1674	2037	2037	2484	1409	1409
	Average life per chick	31.1	36.4	39.2	38.0	40.9	37.7	42.2	42.2	41.6	37.7	42.2	41.6	41.6	38.2	38.2
	Extra days lived per chick	3.9	9.2	12.0	10.8	13.7	10.5	15.0	15.0	14.4	10.5	15.0	14.4	14.4	11.0	11.0
	Units required to live extra day	69	63	75	105	109	159	136	231	173	159	136	231	173	128	128
Control group, basal ration	Chicks in group														233	233
	Units group received														none	none
	Days group lived														6310	6310
	Average life per chick														27.3	27.3

to live an extra day, indicating this solution of carotene in oil to be 71.3 per cent of the potency of the carotene in oil fed in group A.

Group C given carotene in oil of unknown potency was fed on the basis of having the same potency as A, and showed a requirement of 190 A.D.M.A. units to live 1 extra day, indicating this carotene in oil to be 60 per cent of the potency of the carotene in oil fed in group A.

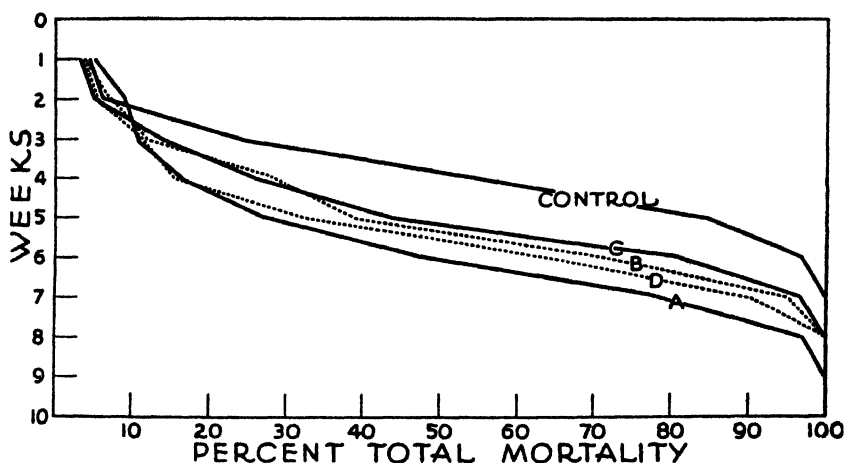


Fig. 1 Mortality curve. Six hundred and twelve White Leghorn chicks. Summary of results. Carotene and vitamin A requirement experiments with 612 day-old White Leghorn chicks. Group A—Carotene in oil—known potency (sample A). Group B—Carotene in oil—unknown potency (sample B). Group C—Carotene in oil—unknown potency (sample C). Group D—Cod liver oil (sample D). Control group, basal ration.

In group D, biologically tested cod liver oil was used in amounts equivalent to that of the carotene in oil of known potency fed to group A. The figure obtained for this group should, therefore, be the same as that of group A. Instead it was 87.9 per cent of the potency of carotene in oil fed in group A. The results were 128 A.D.M.A. units for each extra day, as compared to 114 A.D.M.A. units, or within 10.9 per cent of the expected results.

Figure 1 is a graphic representation of the mortality rate in each group.

Toward the end of the experiment, ten pullets and ten cockerels were selected from the control group. Only chicks that had not yet shown signs of vitamin A deficiency were selected in order to show the weight curve immediately before depletion to the end point which was the death of the chick. They were weighed daily. The weights are recorded on the charts in figure 2 and figure 3. These records are of interest

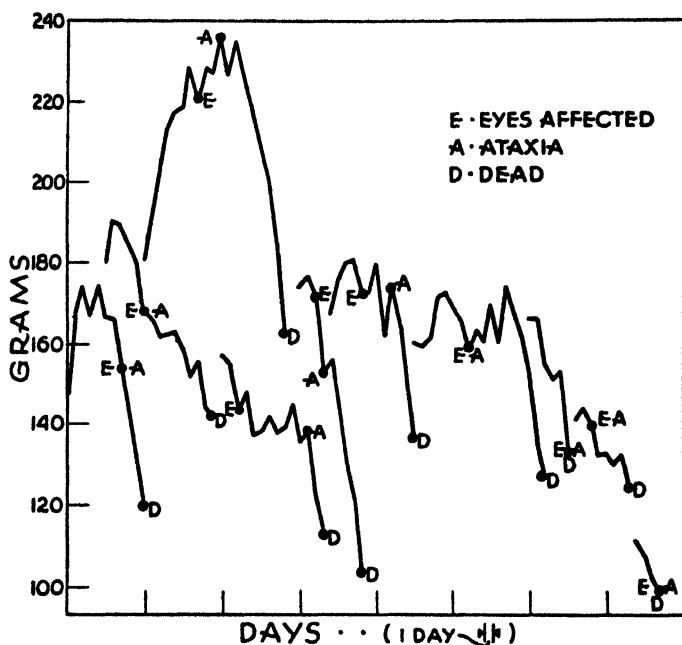


Fig. 2 Weight curves obtained from ten individual White Leghorn pullets. (Daily weighings began at twenty-eighth day from hatching.)

as indicating the type of growth curve during carotene and vitamin A depletion in chicks and the similarity of these growth curves to the growth curves of vitamin A depleted rats. Note the short length of life after the maximum weight has been reached. If chicks are to be used for quantitative tests of vitamin A after depletion, the first signs of ataxia rather than the usual 7 days of stationary or declining weight would be a safer end point to begin giving the curative dose.

For a check that an adequate amount of vitamin D was given in the form of a vitamin D concentrate produced from cod liver oil by the Zucker-Columbia University process, from which the vitamin A was oxidized, tibiae were taken from chicks toward the end of the experiment and were x-rayed. Tibiae examined and shown in plate 1 show normal structure with no evidence of rachitic lesions, thus indicating an ade-

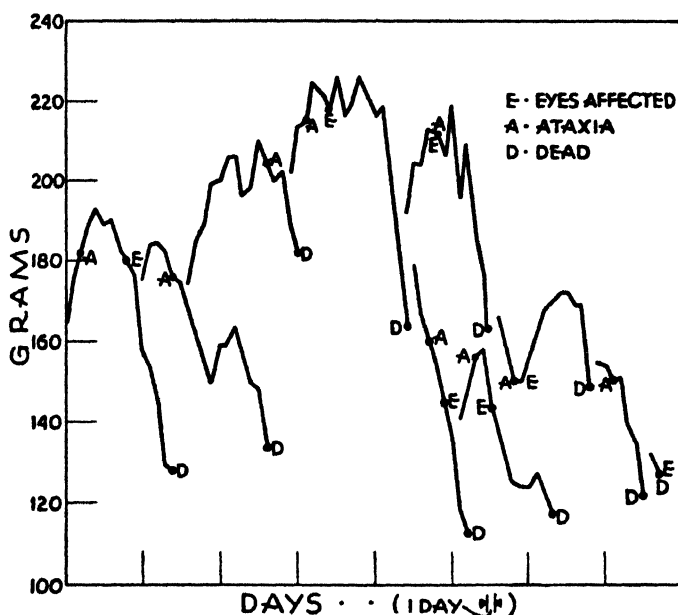


Fig. 3 Weight curves obtained from ten individual White Leghorn cockerels. (Daily weighings began at twenty-eighth day from hatching.)

quate amount of vitamin D. Not one case of 'slipped tendon' was observed in the entire group of 612 chicks. On the other hand, practically all of the 612 chicks in this experiment showed marked ataxia from 3 to 14 days before complete depletion and death. This indicates the inability of an adequate amount of vitamin D to prevent ataxia of this type in carotene and vitamin A depleted chicks. It also suggests the undesirability of using the general term of 'leg weakness' in connection with vitamin D deficiency.

Immediately following this experiment, the same solutions of carotene and vitamin A were used in another entirely unrelated experiment. This indicated a considerable loss of vitamin A in the test solutions at the end of the previous test. While this was not anticipated, it is not at all surprising in view of the fact that the chicks were given the solutions daily over a rather long period. There was considerable exposure to air during this time as the quantity of solution in the bottles decreased with a corresponding increase in the air space in each bottle.

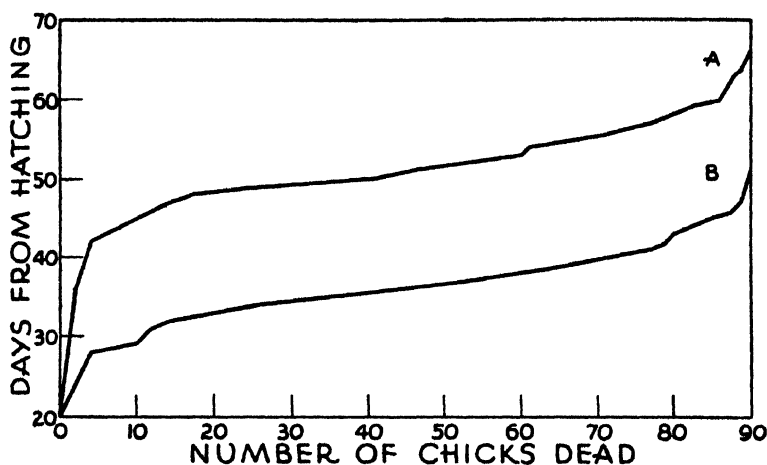


Fig. 4 Mortality curve. a) Ninety chicks each received 1000 A.D.M.A. units of carotene in oil over a period of 8 days beginning with the twentieth day from hatching. b) Ninety controls received the carotene and vitamin A-free basal diet only.

Figure 4 presents data obtained in a separate test carried out for obtaining additional information regarding the length of life of chicks on a diet free from carotene and vitamin A, and in which the possibility of loss of potency would be avoided or minimized by giving the additional vitamin A in a very short period. To study the effect of the addition of carotene in oil to the length of life, 180 day-old White Leghorn chicks were divided into two groups. a) A group of

ninety chicks each received 1000 A.D.M.A. units of carotene in refined cottonseed oil in a period of 8 days, in addition to the same basal diet used in the experiment with the 612 chicks before described. At the end of 8 days the carotene supplement was stopped and the chicks were allowed to deplete. b) A control group of ninety chicks received the carotene and vitamin A free basal diet only. Figure 4 shows the results graphically. The ninety controls lived 3315 days, an average of 36.8 days per chick, while the ninety chicks each receiving 1000 A.D.M.A. units of carotene in oil lived 4662 days, an average of 51.8 days per chick, or an addition of 15 extra days per chick for the group receiving the carotene supplement. This means that the chicks in group A lived on the average 1 extra day for each 66 $\frac{2}{3}$  A.D.M.A. units of vitamin A they received as compared with the control group. Attention is directed to the apparent uniformity of storage of the two groups as evidenced by the parallel mortality curve shown in figure 4. These results are in closer agreement with our preliminary tests by the curative method (Frohring and Jersey, to be published). It will be noted in figure 4 that the addition of the carotene in oil supplement to the basal diet prolonged life and moved the mortality curve upward.

#### DISCUSSION

Provided that these results may be verified, this method might serve as a useful means of comparing the vitamin A content of materials of unknown potency without the necessity of having a rat colony. It is not suggested that this method would be of value in replacing the standard rat unit method, especially where the rat unit method is used for official purposes. It may be useful to laboratories that make only occasional vitamin A tests, and are unable to go to the expense of developing a rat colony suitable for the purpose.

Each time the method was used to assay an unknown, it would be necessary to run both a control group and a 'known potency' group in addition to the groups receiving materials to be tested. The 'known potency' group would be given a

standard carotene in oil solution of known potency. The extra days lived per chick and the units required to live an extra day in the 'known potency' group over that of the average days lived per chick in the control group are used as a basis for determining the approximate amount of vitamin A activity in the unknown material.

Some of the possible advantages of this method might be suggested here. Day-old chicks may be obtained at most any time of the year from recognized hatcheries. The ease of obtaining the chicks means that large numbers, say 100, may be used in each group. This would tend toward accuracy. The equipment required for chicks is comparatively inexpensive compared to that required for rats in that the chicks may be kept on brooders in groups of 100 and the individual cages necessary for rats are avoided. Since death is the end point of the method, as the animals die off the amount of work necessary diminishes rapidly. This method does not involve the weighing of the animals or the use of leg bands, as each group of 100 can be kept in separate brooder compartments.

The principal opportunity for error in this method is the separation of the chicks into groups. Due to different rations used on different breeding farms, there will be variations in the vitamin A activity stored in the egg and consequently in the day-old baby chicks received from these respective hatcheries. Bethke, Kennard and Sassaman ('27) found that eggs from hens fed cod liver oil or having access to blue grass pasture contained approximately five times as much vitamin A as eggs from hens fed the basal diet, which contained 30 per cent yellow corn, or the basal diet supplemented with alfalfa hay. Sherwood and Fraps ('32) estimated from their experimental work that 1 unit of vitamin A in the egg requires 6.3 units in the feed in addition to the maintenance requirements. They also found in four hatching tests that the percentages of eggs hatched increased with the increase in the amounts of vitamin A in the feed, but they were not related to the amounts in the eggs themselves. Either all

chicks from one breeding farm should be used or if the eggs for hatching purposes are obtained from different poultry farms, it would be advisable to divide the chicks from each farm equally into each group. A large number of animals in each group has to be depended upon to avoid the error of this variable. An interesting possibility would be the use of chicks hatched from eggs from the same hen. If uniformity could be obtained by this method, greater accuracy might be obtained with much smaller groups. Experiments along these lines are in progress.

It is interesting to note that the results obtained by this method confirm the report of Sherwood and Fraps ('32) on the relatively high vitamin A requirements of chickens as compared to rats. They say, "The maintenance requirement of White Leghorn pullets weighing about 3.2 pounds is estimated from the experiment at 105 units of vitamin A a day or about 33 units a pound. This is eight times the estimate of 4 units per day per pound for maintenance of growing rats."

Capper, McKibbin and Prentice ('31), in their work with White Wyandottes, have shown that the fowl as well as the rat can convert carotene into vitamin A, and that the vitamin A requirements of the fowl are higher than those of the rat weight for weight.

Kline, Schultze and Hart ('32) have demonstrated that chicks that have been depleted of vitamin A require more than 0.05 mg. of carotene daily in order to grow to maturity. Five-hundredths mg. of carotene would be equivalent to 100 A.D.M.A. units.

It is our opinion that the optimum requirement of the chick is much higher than that indicated by our tests. Based on the results of Green and Mellanby ('31) with the rat that four times the minimum requirement gave the optimum results as far as resistance to infection was concerned, a rather high optimum amount for chicks, probably in the neighborhood of 300 to 400 A.D.M.A. units per day is indicated. It must be borne in mind, however, that our experiments had



to do only with chicks in the age range from day-old up to about 8 to 10 weeks. The requirements for older animals may be entirely different.

The mortality curves of the control chicks fed the carotene and vitamin A-free diet, figures 1 and 4, indicate a wide variation in the amount of vitamin A activity that was carried over from the egg and that enabled the chicks to live for varying lengths of time. In figure 1 the total mortality rate of the 232 controls was 4.3 per cent for the first week (this was approximately the same in groups A, B, C and D for the first week), 6.5 per cent for the first 2 weeks, and 23.3 per cent for the first 3 weeks. By the end of the fourth and fifth weeks, these values increased rapidly to a total of 56 and 85 per cent, respectively. In other words, the total number of animals that died during the fourth and fifth weeks was 62 per cent of the entire group. All the birds were dead by the end of the seventh week. The average survival was 27.2 days. In the ninety controls in figure 4, the chicks lived from 28 to 51 days and averaged 36.8 days per chick which was about 9 days longer than the first group. This difference is assumed to be due to differences in the vitamin A activity storage of the eggs from which the chicks were hatched. The importance of setting up these tests in such a way as to permit the elimination of this variable factor is thus demonstrated. This can be done by running a parallel control group (selected from the same lot of eggs from which the experimental chicks are taken) large enough to give dependable survival figures, which are assumed to be proportional to the vitamin A activity storage of the entire lot of eggs, and which apply, therefore, also to the parallel experimental chick.

Sherwood and Fraps ('32) found that individual hens receiving no vitamin A from yellow corn lived from 34 to over 199 days and commented that it appears to be a reasonable assumption that the length of life of the pullets is a fair measure of the quantity of vitamin A stored by the pullets before the experiment was begun, when they had all the green feed they could eat. If such is the case the amount of vitamin

A stored by different pullets varied to a considerable extent and some may have contained four or five times as much as others. Sherwood and Fraps ('32) are of the opinion that these pullets fed white corn during the experiment would no doubt have died sooner had they been reared with less liberal supply of vitamin A.

Examination of the rations usually recommended and used shows that hens are frequently not supplied with sufficient amounts of carotene and vitamin A when fed without access to green feed. This probably results in many cases in low egg production, low vitamin content of the eggs, undernourishment of the body of the hen, infection and death of the fowl.

From the data here recorded it is apparent that when a ration devoid of carotene or vitamin A or both was fed to day-old chicks the onset of the reaction varied in different chicks, no doubt due to the variations in storage from the egg from which the chicks hatched which in turn was dependent upon the ration of the parent fowl. The addition of carotene or vitamin A to the carotene and vitamin A-free ration delayed the appearance of deficiency symptoms and prolonged life in a rather definite relation to the amount of carotene or vitamin A added to the diet.

#### SUMMARY

1. The minimum vitamin A requirement of the chick is relatively high per pound of body weight as compared to the rat.

2. The minimum requirement of vitamin A of the White Leghorn chick at the age of about 8 weeks is approximately 65 A.D.M.A. units per day.

3. Results were obtained by comparing the average number of days lived per chick in relation to the number of units of vitamin A supplement received as compared to the average of the control group.

4. Chicks offer interesting possibilities as test animals for vitamin A.

5. No cases of 'slipped tendon' were observed in 792 White Leghorn chicks depleted in vitamin A, but given a diet adequate in other respects.

6. Practically all of the 792 chicks depleted in vitamin A showed marked ataxia 3 to 14 days before complete depletion and death, even though given an adequate amount of vitamin D. This suggests the undesirability of using the broad term 'leg weakness' as related to vitamin D deficiency.

7. There is a wide variation in the number of days chicks will live on a vitamin A-free diet, no doubt due to variation in storage from the egg from which the chicks hatched, which in turn was dependent on the storage or ration of the parent fowl. The addition of carotene or vitamin A to the carotene and vitamin A-free ration delayed the appearance of deficiency symptoms and prolonged life in a rather definite relation to the amount of carotene or vitamin A added to the diet.

We are indebted to Mr. F. D. Baird, of the Nutritional Laboratory of the National Oil Products Company, Inc., for making the x-rays of the tibiae, and also to Miss Esther Zurcher for valuable assistance in checking the statistical data.

#### LITERATURE CITED

- BETHEKE, R. M., D. C. KENNAED AND H. L. SASSAMAN 1927 The fat-soluble vitamin content of hen's egg yolk as affected by the ration and management of the layers. *J. Biol. Chem.*, vol. 72, p. 695.
- CAPPER, N. S., I. M. W. MCKIBBIN AND J. H. PRENTICE 1931 Carotene and vitamin A. The conversion of carotene into vitamin A by fowl. *Biochem. J.*, vol. 25, p. 265.
- FROHRING, W. O., AND VERNON JERSEY (To be published.)
- GREEN, H. N., AND E. MELLANBY 1930 Carotene and vitamin A: The anti-infective action of carotene. *Brit. J. Exp. Path.*, vol. 11, p. 81.
- KLINE, O. L., M. O. SCHULTZE AND E. B. HART 1932 Carotene and xanthophyll as sources of vitamin A for the growing chick. *J. Biol. Chem.*, vol. 97, p. 83.
- SHERWOOD, R. M., AND G. S. FRAPS 1932 The quantities of vitamin A required by pullets for maintenance and for egg production. *Texas Agr. Exp. Sta. Bull.*, no. 468.

VITAMIN A REQUIREMENTS OF CHICKS  
W. O. FROHING AND JIRO WAKENO

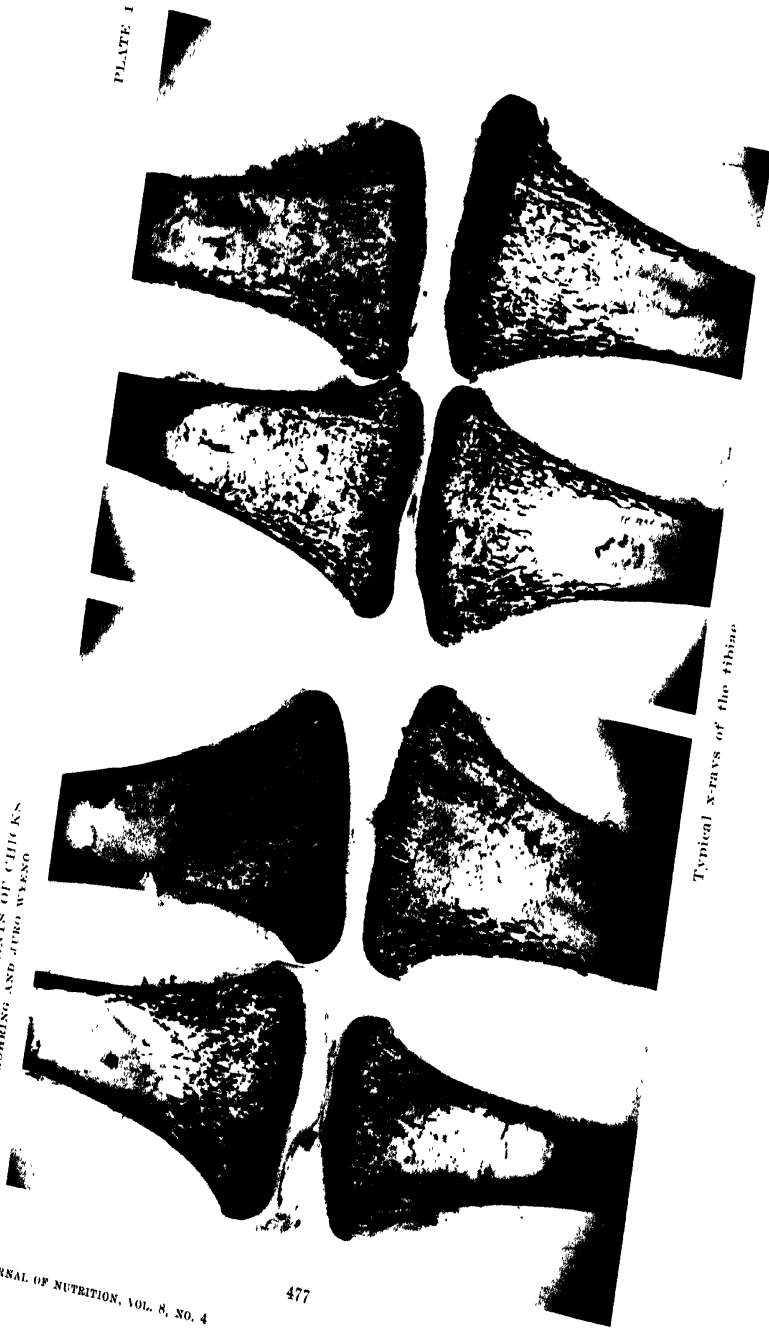


PLATE I

Typical X-rays of the tibiae



# THE EFFECT OF VARIATIONS IN THE DIET ON THE ABSORPTION OF FOOD IN THE ABSENCE OF PANCREATIC DIGESTION

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## ONE FIGURE

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When the pancreatic juice is completely excluded from the intestine the digestion of fat and protein is much disturbed. The experimental evidence of the truth of this statement was furnished by the work of Hess ('08) and Pratt, Lamson and Marks ('09) which has since been confirmed by Brugsch ('19) and Licht and Wagner ('27). It had been known since the early observations of Abelmänn (1890) that total pancreatectomy produced great loss of fat and nitrogen in the stools. After the pancreatic juice is shut off from the intestine the animals usually lose weight rapidly and usually die of inanition unless fresh pancreas is fed.

In spite of the absence of pancreas juice, however, McClure, Vincent and Pratt ('17) observed fairly good absorption of nitrogen and fat for short periods in dogs with occluded pancreatic ducts and in those completely depancreatized.

Falcon-Lesses and Herschenson in this laboratory showed that it was possible to keep dogs deprived of pancreatic juice in good condition for months with no definite loss of weight or strength without the aid of fresh pancreas by feeding definite quantities of milk powder, carbohydrates and yeast. A completely depancreatized dog receiving insulin retained its weight and strength for a year without being fed any

pancreas. These studies have been briefly reported by Pratt ('31).

The present investigation was undertaken to determine the effect of changes in the quantity and quality of the food on the absorption of nitrogen, fat and carbohydrates.

#### METHODS

The three dogs employed in these experiments were operated on by Dr. L. R. Whitaker. A midline incision was made and the mobile duodenum was delivered with the pancreas. A systematic search for pancreatic ducts was made from one end to the other of the corpus pancreatis. Two or three were usually encountered and these were ligated with silk thread both distally and proximally and then severed. Several small blood vessels were ligated, but the remaining blood supply was adequate for the nutrition of the duodenum. The omentum was inserted between the duodenum and the pancreas.

The dogs were kept in metabolism cages throughout the experiments. Absorption periods extended over 3 days and were demarcated by the inclusion of carmine or charcoal in the diet. Feeding took place once a day at 4 P.M. All stools were collected under alcohol and dried first over a water bath and then in a desiccator. Well mixed pulverized specimens were used for analyses and all were done in duplicate.

Nitrogen determinations were performed by the Kjeldahl method. Fats were estimated by the Saxon method ('14) and the percentage of fatty acids was expressed in terms of stearic acid.

For the analysis of the carbohydrate contents we employed an unpublished method developed by Hjort working with Pratt and later modified in this laboratory by Herschenson and Falcon-Lesses. A sample of dried stool is carefully weighed. The sample should be about 2 gm. when the carbohydrate content is suspected to be low, or 1 gm. when high. To the stool specimen in a 500 cc. beaker are added 200 cc. of distilled water and 2 cc. of concentrated hydrochloric acid

(sp.gr. 1.135). This is boiled with a reflux condenser over a sand bath for 2 hours. After cooling, 5 gm. of permutit and 5 gm. of Lloyd's alkaloidal reagent are added and the mixture shaken for 5 minutes. Then 10 per cent sodium hydroxide is added until the preparation is just acid to litmus. By means of a suction filter a clear filtrate is obtained and diluted to volume in a 1000 cc. volumetric flask. The Folin 1929 copper reduction method is carried out on 2 cc. of this filtrate. Careful washings with distilled water must be performed on the contents of the filter as well as when transferring from the beaker to the filter.

The three dogs chosen for these experiments were all females. Following the operation dog 21 and dog 22 were active and hungry on the next day, passing typical 'pancreatic stools' on the third day. Dog 20 refused to eat except for an occasional nibble of finely chopped meat for 3 weeks. She then began to eat well and to gain in weight and strength.

After recovery from the operation the dogs had voracious appetites. The urines, tested with Benedict's qualitative reagent twice a week, showed no sugar except on one or two occasions when only a very slight reduction (green with precipitate) was found. Fasting blood sugars done by the Folin-Malmros method were always below 100 mg. per cent. At no times did the dogs have polydipsia.

Each animal was put on a 3-day metabolism period as soon after the operation as possible. The dogs weighed 7.8 to 9.8 kg. before operation. The composition of the diets used is given in table 1. The daily standard diet consisted of a mixture of 50 gm. of milk powder,<sup>1</sup> 75 gm. of cracker meal, 50 gm. of Valentine dried extracted beef and 5 gm. of Harris brewer's yeast. By several analyses the average food value of this diet during the 3-day period was found equivalent to 245 gm. of carbohydrates, 195 gm. of protein (or 31.2 gm. of nitrogen) and 58.1 gm. of fat, or 2326 Calories. Roughly, this amounts to 95 Calories per kilogram, with 8 gm. protein per kilogram, for each dog daily.

<sup>1</sup> Klim.



TABLE 1

*List of diets used with amounts fed daily*

No. 1	Standard:	Dried milk, 50 gm.; cracker meal, 75 gm.; dried extracted beef, 50 gm.; yeast, 5 gm.
No. 2	Double standard:	Dried milk; 100 gm.; cracker meal, 150 gm.; dried extracted beef, 100 gm.; yeast, 10 gm.
No. 3	Triple standard:	Dried milk, 150 gm.; cracker meal, 150 gm.; dried extracted beef, 150 gm.; yeast, 5 gm.
No. 4	High carbohydrate A:	Dried milk, 25 gm.; cracker meal, 150 gm.; dried extracted beef, 25 gm.; yeast, 5 gm.
No. 5	High carbohydrate B:	Dried milk, 25 gm.; cracker meal, 175 gm.; dried extracted beef, 25 gm.; yeast, 5 gm.
No. 6	High nitrogen A:	Dried milk, 50 gm.; cracker meal, 25 gm.; dried extracted beef, 150 gm.; yeast, 5 gm.
No. 7	High nitrogen B:	Dried milk, 25 gm.; cracker meal, 25 gm.; dried extracted beef, 200 gm.; yeast, 5 gm.
No. 8	High fat A:	Dried milk, 50 gm.; cracker meal, 50 gm.; dried extracted beef, 50 gm.; olive oil, 30 gm.
No. 9	High fat B:	Cracker meal, 75 gm.; 'Nueoa' (cocoanut oil), 100 gm.; dried extracted beef, 50 gm.; yeast, 5 gm.
No. 10	Meat and lard:	Beef heart, 150 gm.; cracker meal, 75 gm.; lard, 20 gm.; bone ash, 10 gm.
No. 11	Large diet of meat and lard:	Beef heart, 400 gm.; cracker meal, 150 gm.; lard, 60 gm.; bone ash, 50 gm.

## EXPERIMENTAL RESULTS

The figures given in table 2 on the dogs without pancreatic digestion while on the standard diet are strikingly different from those on the normal dog taking the same diet which was studied as a control. The increased weight of the feces is an outstanding feature in all the experiments on the operated dogs, the dried feces weighing three to four times as much as those passed by the normal dog. The table shows that the absorption of carbohydrates, protein and fat when compared with the normal is found to be somewhat lessened in every experiment. The absorption of starch is least affected in all and that of nitrogen most disturbed in four out of the

six test periods. The poorest absorption was that of fat, 41.5 per cent, in the experiment on dog 22.

There is no relation shown between the time that elapsed after shutting off the pancreatic juice and the efficiency of absorption. The pancreas atrophies rapidly and if the gland exerted any influence on digestion and absorption after all the ducts are occluded by the passage of the digestive ferments into the blood and thence through the bile into the intestine, as the work of Zucker, Newburger and Berg ('32) suggested, this would be expected to lessen as the pancreas

TABLE 2

*Absorption studies with the standard diet on dogs deprived of pancreatic digestion*

DOG	NUMBER OF EXPERIMENT	TIME AFTER OPERATION	DRY WEIGHT OF FECES IN-GRAMS		ABSORPTION		
			Test period	Per day	Cho per cent	N per cent	Fat per cent
21	1	1 week	120	40	95.8	60.0	73.1
21 <sup>1</sup>	6	7½ months	148	37	97.3	57.1	56.0
20	1	1 month	169	56.3	83.5	56.4	60.3
20	6	5½ months	114	38	97.3	55.9	86.9
20 <sup>1</sup>	7	10½ months	204	51	95.2	47.0	67.8
22	1	2 weeks	170	56.7	92.2	56.7	41.5
Normal dog as control	1	....	37	12.3	99.0	91.2	91.3

<sup>1</sup> Metabolism periods carried over 4 days instead of 3 days.

shrunk. At the end of 4 or 5 months, we have found it reduced to a narrow strand not weighing more than a few grams. The pancreas of dog 21, killed 8 months after the separation of the pancreas from the duodenum, was found to be only 4 cm. long, 1 cm. wide and 2 mm. thick. Dog 20, however, showed better absorption of starch and fat 10 months after the operation than she did after 1 month; and the feces passed daily during the test weighed less. There is no evidence, however, that the stomach or intestine compensates for the lack of pancreatic digestion with the passage of time. This is shown by the studies on dog 21. She absorbed less nitrogen and fat 7 months after the operation than at the end of the first week.

Of our three dogs, dog 22 seemed to recover from the effects of the operation more quickly than the others. She was kept in a large animal room in another department and was fed 150 gm. of ground beef heart, 75 gm. of cracker meal, 10 gm. of lard and 5 gm. of bone ash each day. The dog received yeast for only 6 days. During the first month following the operation she dropped from 9 kg. in weight to 8 kg., but maintained her strength and normal state of activity. However, after another month the effects of inanition were obvious, although the unoperated dogs in the same room thrived. She became so feeble during the last 2 weeks of her life that she could barely walk, and her appetite, which was formerly voracious, became very poor. Three months after operation she was found dead. Her weight had fallen to 6.0 kg.

In contrast to dog 22, dogs 20 and 21 were kept in our own animal room and received milk powder and yeast in their diet. These animals remained in a healthy and strong condition. An attempt was made to keep the weight near the preoperative level by varying the quantity of food in the diets each week or every other week. The mechanism of maintaining their weight was very labile. When kept on the above-mentioned standard diet they tended to lose weight and showed a desire for more food.

The figure shows the weight responses during a period of a month and a half, to the standard diet and to one almost three times its size. After having been kept on a small diet for a length of time, the animals ate the large diets greedily for about a week to 10 days, when their appetites began to decrease and they rejected more than half of the food offered them. Parallel to this they gained weight at first on the large diet, but seemed better when put back on the standard diet. We carried dog 20 through three such cycles in addition to the one shown in the chart with similar results. No single optimum diet was found, but the amount of food was changed dependent on the weight and condition of the dog. In this way the animals were kept in good shape throughout the months of study.

In order to find the cause of the increased weight of the dogs when on large diets, absorption studies were carried out during those periods. It was found that large quantities of the additional food were absorbed and the general condition of the animals improved. These results are tabulated in tables 3, 4 and 5, where the food values of the various diets are given with the percentage of the food absorbed during the 3-day metabolic periods to serve as an index of the efficiency of absorption when on the various diets.

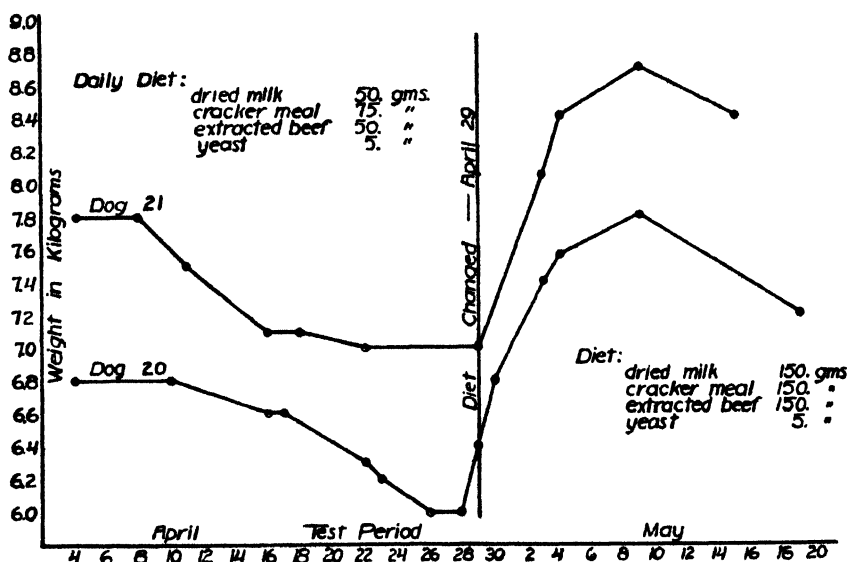


Fig. 1 The effect of an increase of diet on the weight of the dogs deprived of pancreatic external secretion.

In table 3 are summarized the results of ten absorption experiments on dog 20. These observations extended over a period of 11 months. Although no pancreas was fed, this dog without any pancreatic digestion remained in good condition after recovery from the operation. Preoperative weight was 7.0 kg. Operation July 22, 1931. Weight in June, 1933, was 7.2 kg. The dog was then in excellent condition nearly 2 years after the operation. She died February 4, 1934, of acute entero-colitis. The atrophied pancreas formed

a narrow cord 4 cm. long. The ability of this dog deprived of pancreatic digestion to live and remain in good condition for many months without the aid of raw pancreas in the diet confirms the results reported by Pratt ('31). In previous work Pratt had found that dogs deprived of pancreatic secretion when allowed to eat freely usually failed to absorb about 50 per cent of the nitrogen and fat of their food. The result was a slowly progressive loss of weight ending in death from inanition unless fresh pancreas was given. Many other ob-

TABLE 3

*Absorption experiments on dog 20. Preoperative weight 7.8 kg. Pancreas separated from duodenum July 22, 1931*

NUMBER OF EXPERI- MENTS	DATE	DIET				FECELS						
		Number and type	Food value for 3 days in grams			Dried weight, grams	Percentage composition			Percentage absorbed		
			Cho	N	F		Cho	N	F	Cho	N	F
1	Sept. 1-3	No. 1 standard	245.5	31.2	58.0	169	23.9	8	13.6	83.5	55.8	60.3
2	Sept. 29- Oct. 2	No. 4 high carbo- hydrates A	396.0	21.7	32.5	70	21.5	7.81	4.25	96.2	74.9	90.8
3	Oct. 5-8	No. 6 high nitrogen	125.7	68.98	84.3	161	0	12.81	4.56	100	71.5	91.3
4	Oct. 13-15	No. 8 high fat	185.4	29.95	147.3	99	4.4	11.3	9.4	97.7	62.7	93.7
5	Oct. 19-21	No. 4 high carbo- hydrates A	515.7	24.28	33.9	127	30.7	7.87	5.25	92.6	58.8	79.9
6	Nov. 16-18	No. 1 standard	245.4	31.2	58.0	114.5	6.25	12.05	6.65	96.9	55.8	86.9
7	Apr. 25-28	No. 1 standard	327.2	41.6	77.4	204	7.7	10.8	12.2	95.2	47.0	67.8
8	May 23-26	No. 3 triple standard	538.5	88.2	171.9	531	9.3	9.45	13.6	90.8	43.1	58.0
9	June 30 to July 2	No. 11 large diet meat and lard	358.5	59.5	256.5	592	16.8	3.8	26.4	72.2	62.6	39.6
10	July 9-11	No. 10 meat and lard	179.4	23.2	89.1	242	21.6	5.6	15.0	70.8	41.8	59.3

servers have found that dogs devoid of pancreatic secretion died within a short period of time unless given pancreas in their food. The usual diet was beef, to which sometimes lard or other fat was added. Penau and Simonnet ('26), however, kept a depancreatized dog receiving insulin, alive and in good condition for more than 2 years without feeding raw pancreas or pancreatic preparations. They attribute their success to the use of sugar in the diet and their method of feeding. The dog was given six small meals a day with an interval of 2 hours between meals. At each feeding it re-

ceived 20 gm. of beef, 10 gm. of sugar, 50 cc. of fresh milk, 20 gm. of bread, and 5 gm. of powdered bone. For an evening meal 200 gm. of meat were given. No absorption studies were made. Our dogs that lived for many months received 50 gm. or more of milk powder as a part of their regular diet and 5 gm. of yeast powder daily. They were fed only once a day.

Two special metabolism periods were included in our studies to give a basis of comparison with the results obtained by Pratt, Lamson and Marks ('09). The diet similar to that used by these workers consisted of beef heart, cracker meal and lard, to which bone ash was added. The results are given under experiments 9 and 10 in table 2. The stools were unusually heavy. When large amounts of beef and lard were fed (experiment 9) less fat was utilized by this dog than in any other test-period. In the experiment with the smaller amounts of beef and lard the percentage of nitrogen absorbed was lower than in any other test. The findings agree with the earlier work published from the Harvard laboratory.

During some metabolic periods (experiments 2, 3 and 4) dog 20 showed such excellent absorption of both carbohydrates and fats that one might have doubted whether all the pancreatic juice had been excluded from the intestine had not studies before as well as afterward shown the typically diminished absorption usually obtained in animals deprived of pancreatic juice.

The studies on dog 21 (table 4) were made for the most part simultaneously with those on dog 20 using the same diets, but the percentage of absorption was, as a rule, lower. The condition of the dogs also showed a decided contrast at the times the earlier tests were made. Dog 20, more accustomed to laboratory life, was placid, ate with good appetite, but not voraciously, usually gained and lost weight only slowly when the diets were increased or decreased, respectively, and seemed to be in the best of health. Dog 21 was decidedly unhappy with confinement to a cage; bolted all her food, showed very labile weight responses with changes in the size of the

diets, and seemed to lose considerable strength. This weakness, however, was temporary, lasting about 2 months.

From the comparative study of these 2 dogs over a period of 8 months it is clearly evident that unknown factors cause such great variation in absorption that it is very difficult to compare the value of different diets. When dog 20 was given a high carbohydrate-low fat diet the bulk of the feces was

TABLE 4

*Absorption experiments on dog 21. Preoperative weight 9.8 kg. Pancreas separated from duodenum October 7, 1931*

NUMBER OF EXPERI- MENTS	DATE	DIET				FECES							
		Number and type	Food value for 3 days in grams			Dried weight, grams	Percentage composition			Percentage absorbed			
			Cho	N	F		Cho	N	F	Cho	N	F	
1	Oct. 11-13	No. 1 standard	245.4	31.2	58.1	120	8.6	10.4	13.0	95.8	60.0	73.2	
2	Oct. 19-22	No. 5 high-carbo- hydrate B	455.7	23.0	33.2	279	46.6	5.6	9.3	71.3	75.1	22.0	
3	Nov. 12-15	No. 7 high nitrogen B	97.2	85.9	77.1	358	2.8	12.5	10.8	87.7	47.8	49.9	
4	Dec. 8-10	No. 9 high fat B	188.4	24.8	264.9	190	7.4	7.8	29.8	97.1	68.5	88.7	
5	Dec. 21-23	No. 3 triple standard	538.5	88.2	171.9	699	28.1	7.8	17.7	63.5	38.2	30.3	
6	April 25-28	No. 1 standard	327.2	41.6	77.4	148	5.8	12.1	23.0	97.3	57.0	56.1	
7	May 24	No. 3 triple standard	179.5	29.4	57.3	178	9.1	8.8	21.8	90.9	47.0	32.5	
	Killed at end of 24 hours. Contents of colon added to feces												

small and the food well utilized in two experiments. Despite the very good results obtained with this diet in these two periods one cannot conclude that it is an excellent diet for a dog without pancreatic digestion, since when it was fed to dog 21 (experiment 2) there was very poor utilization. The analysis showed that no less than 46.6 per cent of the dried stool was composed of carbohydrates and the absorption of fat in this experiment, 22.0 per cent, was the lowest obtained in the entire series.

The striking feature in the study of dog 22 is the apparent relation between poor absorption and the poor condition of the animal. During the interval of 2 weeks between the operation and the first test period the dog (table 5) was fed no milk, fat or yeast. The diet consisted of beef heart, cracker meal and lard. The fact that she had greater weight of feces and poorer absorption of fat when placed on the standard diet (table 5, experiment 1) than the other two dogs was possibly due to the absence of milk and yeast from her previous diet. She was losing weight at the time of the test. In the

TABLE 5

*Absorption experiments on dog 22. Preoperative weight 9.2 kg. Pancreas separated from duodenum February 9, 1932*

NUMBER OF EXPERI- MENTS	DATE 1932	DIET				FECES						
		Number and type	Food value for 3 days in grams			Dried weight, grams	Percentage composition			Percentage absorbed		
			Cho	N	Fat		Cho	N	Fat	Cho	N	Fat
1	Feb. 24-26	No. 1 standard	245.4	31.2	58.1	170	10.9	8.0	20.0	92.4	56.4	41.5
2	Mar. 7-9	No. 2 Double standard	490.8	62.4	116.2	405	10.9	8.9	21.3	91.0	42.3	25.7

second experiment when given twice the usual standard diet the utilization of starch, protein and fat was still less. The dog continued to fail and died apparently of inanition.

*Absorption of carbohydrates.* When only 126 gm. of starch were fed to dog 20 (experiment 3), and that in the form of cracker meal and dried milk, the carbohydrates were completely utilized. Dog 21, however, when given a similar diet absorbed only 87.7 per cent of the starch. When given a very large amount of starch, he showed still poorer absorption, namely, 71.3 per cent. The poorest utilization of starch in our study was 63.5 per cent. This result was obtained on dog 21 when fed three times the standard diet (experiment 5). When given the same diet later, he absorbed 90.9 per cent of the starch! The results of these two experiments show



clearly that the absorption of starch is largely influenced by factors independent of the diet.

The importance of feeding carbohydrates to dogs deprived of their pancreatic secretion has not been sufficiently recognized. Its absence from the diet may explain in part the rapid emaciation and speedy death of animals often observed after obstructing the flow of pancreatic juice into the intestine. Our dog 22, however, lost weight and finally died of inanition, although fed on an abundance of carbohydrates. Brugsch ('19) fed his dogs, in which all pancreatic ducts had been tied, horse meat and beef suet. One lived only 3 weeks and the other 5 weeks. It should be emphasized that carbohydrates in any diet are well utilized in the absence of pancreatic digestion.

*Absorption of nitrogen.* On a well-balanced diet the absorption of protein is usually more disturbed in the absence of pancreatic digestion than that of fat or carbohydrates. The amount of nitrogen found in the stools of a normal dog taking 150 gm. of beef heart, 70 gm. cracker meal, 10 gm. of lard and 5 gm. of bone ash, in a 3-day experiment, was 2.1 gm., the percentage absorbed being 90.8. In an observation on another normal dog taking the standard diet the percentage of nitrogen absorbed was 91.2. When dog 20 was given exactly the same amount of food the nitrogen in the stools was 13.8 gm., the percentage absorbed dropping to 55.8 per cent. The smallest amount found in the feces in any of the studies on these dogs was 5.5 gm. The percentage absorbed was 74.9. This was on a high carbohydrate diet. The poorest nitrogen absorption was shown by dog 21 when given three times the standard diet. It was 38.2 per cent.

*Absorption of fat.* McClure, Vincent and Pratt ('17) found that their depancreatized dogs absorbed 17.6 to 45.3 per cent of fat when given butter or lard. Their experiments proved that, under certain conditions in the absence of the pancreas, fat may be absorbed from the intestines in relatively large amounts, ranging from 19.8 to 179.4 gm., during the experimental periods of 3 to 5 days, even when the fat fed is not

in the form of an emulsion. These experiments were made before the discovery of insulin and as a result the dogs had an unchecked fatal diabetes.

Ten experiments were made on dog 20 to determine the influence of variations in the diet on the absorption of fat. Although her pancreas was present and she did not have diabetes, in no experiment did she absorb fat as well as did the depancreatized dog in the above test.

Marked variations in absorption of fat occur independently of the diet, as is clearly shown by the results in three experiments on dog 20 when the same standard diet was given. In September 60.3 per cent of fat was utilized, in November 86.0 per cent and in the following April 67.8 per cent. The first two experiments on dog 21 likewise show that absorption cannot be predicted from knowing the food fed and they illustrate our inability to find an optimum diet. In October, when given the standard diet, 73.2 per cent was absorbed, while dog 20 absorbed only 60.3 per cent of the fat in this diet. As dog 20, a few weeks later, absorbed 90.8 per cent of the fat in the high carbohydrate diet, it seemed logical to expect that dog 21 would do as well or better. On this diet, however, she absorbed only 22 per cent of the fat! This was the poorest utilization of fat observed in our studies.

Dog 22 was in poor general condition and absorbed only 41.5 per cent of the fat on the standard diet in contrast to 60.3, 86.9 and 67.8 per cent by dog 20, and 73.2 and 56.1 per cent by dog 21 on the same diet.

It is evident that in dogs deprived of pancreatic digestion the absorption of fat is largely dependent on factors other than diet. It was clearly shown that milk fat was better absorbed than lard. This is not always the case, as one of the dogs studied by McClure, Vincent and Pratt (dog 3, experiments 3 and 4) absorbed 27.2 per cent when the fat fed was in the form of butter and 37.1 per cent when in the form of lard. The best absorption of fat observed in the present studies was on a high fat diet (table 2, experiment 4), but as an absorption of 90 per cent or more was also obtained on high

carbohydrate and high nitrogen diets it is evident that the proportion of the various food stuffs is not an important factor in determining the absorption of fat. A diet rich in carbohydrates and low in fat (table 2, experiment 2) was well utilized and 90.8 per cent of the fat absorbed. Almost exactly the same small amount of fat was given a few weeks later in a similar combination and only 79.9 per cent of the fat was absorbed.

#### SUPPLEMENTARY OBSERVATIONS

Practically all of the early workers on this subject neglected to include vitamins in the dogs' diets. That this factor is important can be readily seen in the published protocols of Hershey and Soskin ('31) where avitaminosis was commonly encountered in depancreatized dogs. In our experiments 5 gm. of Harris brewers' yeast were included in the diet of each dog daily. In spite of this, dog 21 showed signs of a lack of vitamins; toward the end of our experiments she developed a spasticity of her hind limbs, and began to grow progressively weaker. After increasing the vitamins in her diet, she regained her former strength, but retained a residual spasticity in her hind legs. Yeast has been shown by Nasset, Pierce and Murlin ('31) to have no effect on the amount of nitrogen excreted through the feces in depancreatized dogs. Its administration, however, was followed by a definitely increased retention of nitrogen in the body, but unaccompanied by any corresponding increase in weight. As the histological studies of Mottram, Cramer and Drew ('22) indicate that vitamins hasten the absorption of fat, it may be that the presence of yeast in the diet in our experiments is a possible contributory factor in producing the fairly good absorption of fat. However, this view is rendered improbable by the fact that Nasset, Pierce and Murlin's dogs failed to gain weight during the periods when yeast was included in the diet.

It must be emphasized that, although our experiments show that fairly good absorption is possible when all the pancreatic ducts are ligated, the stools still showed most of the character-

istics due to the absence of pancreatic juice. During our experiments we always had large bulky stools with an absolute increase in the dry weight. When the diets were large, starch granules, particles of undigested beef, and fat droplets could easily be found by the usual microscopic methods. At times these were not all present in a single stool specimen in sufficient quantities to be readily demonstrated, but at least one, and usually two of the three food constituents could be found in the feces.

The largest portion of fats in the stools (45 per cent to 60 per cent) was found split in the form of fatty acids and soaps. This is a confirmation of the results of earlier experimenters. When olive oil and cocoanut oil were fed (tables 3 and 4) a large percentage was found to be absorbed in each case.

#### DISCUSSION OF RESULTS

From the data presented it can be readily seen that a very large amount of carbohydrates was absorbed by the dogs when the diets were rich in starch. The absorption of nitrogen and fat was much less, both relatively and absolutely. The largest amount of nitrogen utilized was 41.5 gm., but this was only 47 per cent of the nitrogen of the diet.

The greatly increased absorption on the large diets occurred in the face of much waste and poor efficiency, as was evidenced by the fact that much more of the ingested food was found in the stools when the large diets were fed. During the same periods represented in chart 1, while on the standard diet dog 21 passed 460 gm. in 4 days, whereas on the large diet she defecated 575 gm. in 1 day. Dog 20 during the corresponding periods passed 611 gm. in 4 days in contrast to 1430 gm. in 3 days when the larger quantity of food was given. This waste is also manifested when the percentage of food absorbed is calculated. Despite the fact that the actual amount of food absorbed was larger with the larger diets, the percentage of carbohydrates, nitrogen, and fats absorbed dropped, in the case of dog 21 from 96.5 per cent to 90.9 per

cent, from 58.4 per cent to 47.0 per cent, and from 64.7 per cent to 32.5 per cent, respectively, and in the case of dog 20 from 96.2 per cent to 90.8 per cent, from 51.4 per cent to 43.1 per cent, and from 77.3 per cent to 58.0 per cent, respectively. This same behavior was observed in a similar experiment with dog 22.

#### CONCLUSIONS

1. Dogs with pancreatic juice wholly excluded from the intestine can be made to absorb large quantities of food, including fats, even though a high percentage of the nitrogen and fat of the diet may appear in the stools. The carbohydrates fed are usually well absorbed in the absence of pancreatic digestion.

2. These dogs can live for long periods of time in a relatively healthy condition without the addition of fresh pancreas to the diet.

3. They can be made to gain weight and absorb increased amounts of food when their diet is increased, but this is done with impaired efficiency, the animals losing more in their stools. Also, the dogs cannot tolerate these large diets over a long period of time.

4. The improved absorption is not related to variations in the size of the carbohydrate, protein, or fat fractions of the diet.

5. Not only do different dogs deprived of pancreatic digestion absorb different amounts of starch, nitrogen and fat on the same diet, but the same dog on the same diet at different times may absorb very different amounts. From these findings it is evident that unknown factors influenced profoundly the absorption of food in our dogs.

6. The better absorption and the better general health of our dogs compared with those reported by most workers seem due chiefly to the standard diet we employed which contained a liberal amount of carbohydrates, milk fat and vitamins.

## LITERATURE CITED

- ABELMANN, M. 1890 Ueber die Ausnutzung der Nahrungstoffe nach Pankreas-exstirpation mit besonderer Berücksichtigung der Lehre von der Fettresorption. Inaugural Dissertation, Dorpat.
- BRUGSCH, T. 1919 Aeusserere Pankreasfunktion und Pankreasdiagnostik. *Zeitschr. f. exp. Path. u. Ther.*, Bd. 20, S. 473.
- HESS, O. 1908 Experimentelle Beiträge zur Anatomie und Pathologie des Pankreas. *Med. Naturwiss. Arch.*, Bd. 1, S. 161.
- LICHT, H., AND A. WAGNER 1927 Gibt es ein die Fettresorption förderndes inneres Sekret des Pankreas? (Ueber den Einfluss des Insulins auf die Fettresorption). *Klin. Wochenschr.*, Bd. 6, S. 1982.
- MCCLOURE, C. W., B. VINCENT AND J. H. PRATT 1917 The absorption of fat in partially and in completely depancreatized dogs. *J. Exp. Med.*, vol. 25, p. 381.
- MOTTRAM, J. C., W. CRAMER AND A. H. DREW 1922 Vitamins, exposure to radium and intestinal fat absorption. *Brit. J. Exp. Path.*, vol. 3, p. 179.
- NASSET, E. S., H. B. PIERCE AND J. R. MURLIN 1931 The influence of yeast on protein metabolism in normal and depancreatized dogs. *J. Lab. and Clin. Med.*, vol. 16, p. 1151.
- PENAU, H., AND H. SIMONNET 1926 Insuline et diabète expérimental. *Ann. de méd.*, T. 19, p. 401.
- PRATT, J. H. 1931 The diagnosis of chronic pancreatic disease. *Intern. Clinics.*, Philadelphia, 41st series, vol. 3, p. 164.
- PRATT, J. H., P. D. LAMSON AND H. K. MARKS 1909 The effect of excluding pancreatic juice from the intestine. *Trans. Assoc. Am. Phys.*, vol. 24, p. 266.
- SAXON, G. J. 1914 A method for the determination of the total fats of undried feces and other moist masses. *J. Biol. Chem.*, vol. 17, p. 99.
- ZUCKER, T. F., P. G. NEWBURGER AND B. N. BERG 1932 The amylase of serum in relation to functional states of the pancreas. *Am. J. Physiol.*, vol. 102, p. 209.



# A STUDY OF MANGANESE RETENTIONS IN CHILDREN

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Recent studies by Skinner, Peterson and Steenbock ('31), Orent and McCollum ('31), and Kemmerer, Elvehjem and Hart ('31) have confirmed the earlier hypotheses of McHargue ('26) and McCarrison ('27) to the effect that manganese is an essential constituent of the animal organism. Rations containing less than the minimum amounts result in impotency in rats (Orent and McCollum), congenital debility in the young,<sup>1</sup> and possibly a failure in mammary gland development in the parturient female (Orent and McCollum). The addition of manganese to rations lacking in this element resulted in a marked increase in retention (Skinner, Peterson and Steenbock), especially during fetal life; indeed, the storage of manganese during fetal development as well as in the postnatal period seems to be of signal importance to the developing organism. But, apart from the fact that diets lacking in manganese result in testicular atrophy, the specific role it plays in the well-being of the organism as well as the relative amounts needed in the various phases of the life cycle are unknown. In rats the margin of safety in food appears to lie between 1.7 and 2.3 mg. per 100 calories.<sup>1</sup>

Since the manganese content of the more usual foods used by man differs so widely, ranging from the small amounts in milk (average 0.03 mg. per liter) and protein foods in general, to much higher levels in certain vegetables and whole

 Unpublished data.



grain cereals, it is possible that some diets consisting largely of milk and refined cereals, used more largely for children, may contain too little. It therefore seemed desirable to determine the amount of manganese retained by children at various levels of ingestion.

#### EXPERIMENTAL PROCEDURE

The study was made with seven children, three girls and four boys, ranging in age from 3 to 5 years. Each observation included a preliminary period of at least 7 days, and with one exception, a metabolism period of 10 days. From the beginning, the children received a constant weighed diet. The procedure in the care of the children, preparation of food and collection of stools and urine were the same as that reported in a previous study from this laboratory (Daniels and Wright, '34).

Three diets containing different amounts of manganese were selected for study, the choice of food being based on the published analyses of Hodges and Peterson ('31), Peterson and Skinner ('31), and Remington and Shiver ('30). The low and intermediate manganese diets (diets A and B) differed only in the choice of cereal foods, white bread and (with one exception) oatmeal being used in the former, and with the latter commercial whole wheat bread and a whole wheat breakfast cereal. In the high manganese diets (diet C) besides the whole grain cereal foods, high manganese-containing fruits and vegetables, blueberries, pineapple and beets were substituted for those containing the lesser amounts in the other two diets, namely, carrots, apples and prunes. All three diets contained milk, meat, eggs, bananas, potatoes, tomatoes, orange juice and cod liver oil.

The manganese in the food and stools was determined by the method of Davidson and Capen ('29). One day's aliquot of the food for each child, and the stools for the entire metabolism period were dried to constant weight on the water-bath and subsequently in a Freas oven, ground in a Pyrex mortar, and ashed in an electric furnace, such amounts of

the dried material being used as would give satisfactory readings when matched with standards containing from 0.2 to 0.25 mg. of manganese. Triplicate determinations were made in all cases, a known amount of manganese being added to the third sample to test the technic by recovery. In the few instances where recoveries fell below 93 per cent, the determinations were repeated. Distilled water and all reagents used in the analyses were found by test to contain no manganese.

Children's urines are so low in manganese that it was found necessary to make the tests with the pooled 10-day excretions. After thorough mixing, the total amount was divided in halves and these duplicates were concentrated as far as possible on the steam bath, heated repeatedly with concentrated nitric acid until the organic material was driven off, and subsequently treated with repeated amounts of concentrated sulphuric acid to remove the chlorides. The residues were then dissolved in water, the supernatant fluid decanted, and the process repeated a second time. The total solutions were concentrated to 100 cc., 0.3 gm. of potassium periodate was added to each, and enough solid sodium hydroxide to bring the mixtures to more nearly the neutral point. These were then brought to the boiling point and matched with standard permanganate solutions. Duplicate determinations were thus made with all urines.

## RESULTS

The manganese content of the various diets taken by the children shows considerable variation—from 0.101 mg. per kilogram of body weight at the low ingestion level, to 0.304 mg. per kilogram at the high level (table 1). Of this the larger proportion was excreted by way of the gut. The percentage of ingested manganese excreted in the stool (79 per cent) was the same with the two diets (diets B and C) containing the larger amounts of manganese. At the lower ingestion level (diet A) a considerably larger percentage (92 per cent) was excreted. This was somewhat surprising,

since it was anticipated that the diets containing the larger amounts of manganese might result in a lower percentage of absorption because of the amount of roughage contained therein. The alimentary excretion would seem to be in part metabolized manganese, as well as that which was unabsorbed. The manganese of the urine was exceedingly small, depending

TABLE 1  
*The ingestion, excretion and retention of manganese*

NAME	DATE	AGE	WEIGHT	INTAKE		OUTPUT			RETENTION	
				Total	Per kilo-gram	Urine	Feces	Total	Total	Per kilo-gram
Diet A. Low in Manganese										
		<i>months</i>	<i>kg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
D.B.	1/10	64	18.62	1.885	0.101	0.006	1.690	1.696	0.189	0.010
L.B.	1/10	61	15.54	1.888	0.121	0.007	1.761	1.768	0.120	0.008
V.L.	1/10	60	16.19	1.890	0.117	0.007	1.696	1.703	0.187	0.012
B.S.	10/24	50	15.00	1.690	0.113	0.008	1.518	1.526	0.164	0.011
Average		59	16.36	1.836	0.113	0.007	1.666	1.673	0.165	0.010
Diet B. Intermediary in Manganese										
B.S.	1/30	53	14.35	2.151	0.150	0.007	1.792	1.799	0.352	0.024
L.B.	2/1	62	16.19	2.265	0.140	0.009	2.036	2.045	0.220	0.014
P.H.	1/30	44	16.56	2.221	0.134	0.007	1.890	1.897	0.334	0.020
F.G.	10/24	52	14.52	2.502	0.172	0.010	1.967	1.977	0.525	0.036
J.P.	10/24	46	17.63	3.435	0.195	0.013	2.350	2.363	1.072	0.061
Average		51	15.85	2.515	0.158	0.009	2.007	2.016	0.501	0.031
Diet C. High in Manganese										
D.B.	12/11	63	17.97	5.226	0.291	0.013	4.077	4.090	1.136	0.063
L.B.	12/11	60	15.71	4.976	0.317	0.012	4.103	4.115	0.861	0.055
V.L.	12/11	59	15.54	4.873	0.314	0.012	3.936	3.948	0.925	0.060
Average		61	16.40	5.025	0.307	0.012	4.038	4.051	0.974	0.059

in part on the intake, the lowest amount (0.006 mg.) being co-existent with the lowest intake and the larger amounts (0.012 mg.) correlating with the higher intakes.

The amount of manganese retained in the studies herein reported was found to be higher at the higher ingestion levels; for example, at an ingestion of 0.11 mg. per kilogram

the average retention was 0.010 per kilogram, whereas 0.059 mg. per kilogram was retained with an average ingestion of 0.307 mg. That the amount retained is fairly proportional to the amount ingested, within the limits of the study, is further indicated by the retentions of four children: L.B., who was studied at the three levels; and V.L., B.S. and D.B., who were studied during two ingestion levels. In all cases, more manganese was retained with the higher ingestions. For example, L.B. retained 0.008 mg., 0.014 mg. and 0.055 mg. per kilogram at ingestions of 0.121, 0.140 and 0.317 mg. per kilogram, respectively, while V.L. retained 0.012 mg. and 0.060 mg. per kilogram with ingestions of 0.117 and 0.314 mg. per kilogram. J.P., on the other hand, retained as much manganese at an ingestion level of 0.195 as other children at somewhat higher levels. His diet at the intermediary manganese level consisted of a larger proportion of whole wheat cereal than that of the other children of diet B group, thus making for a larger ingestion of manganese and a correspondingly higher retention.

The variations in the manganese retentions of the children studied at the several levels may be due in part to their previous store and in part to the difference in their individual physiologic needs. But the fact that consistently higher retentions were obtained at the higher ingestion levels in children who had received fair amounts previously would seem to indicate that there is a definite manganese need which may not be met by diets which appear adequate from other standpoints. In the age group studied this would seem to lie between 0.20 and 0.30 mg. per kilogram.

#### SUMMARY

Twelve manganese balance studies with seven children have been made at three different levels of ingestion. Within the limits of the study the amount retained was found to be proportional to the amount ingested, thus indicating that manganese is essential to the physiological development of children. It is suggested that the diet for children should contain between 0.20 and 0.30 mg. of manganese per kilogram.

## LITERATURE CITED

- DANIELS, A. L., AND O. E. WRIGHT 1934 Iron and copper retentions in young children. *J. Nutrition*, vol. 8, p. 125.
- DAVIDSON, J., AND R. G. CAPEN 1929 The determination of manganese in plant materials by the periodate method. *Assoc. Official Agr. Chem.*, vol. 12, p. 310.
- HODGES, M. A., AND W. H. PETERSON 1931 Manganese, copper and iron content of serving portions of common foods. *J. Am. Dietetics Assoc.*, vol. 7, p. 6.
- KEMMERER, A. R., C. A. ELVEHJEM, AND E. B. HART 1931 Studies on the relation of manganese to the nutrition of the mouse. *J. Biol. Chem.*, vol. 92, p. 623.
- MCCARRISON, R. 1927 The effect of manganese on growth. *Indian J. Med. Research*, vol. 14, p. 641.
- MCHARGUE, J. S. 1926 Further evidence that small quantities of copper, manganese and zinc are factors in the metabolism of animals. *Am. J. Physiol.*, vol. 77, p. 245.
- ORENT, E. R., AND E. V. MCCOLLUM 1931 Effects of deprivation of manganese in the rat. *J. Biol. Chem.*, vol. 92, p. 651.
- PETERSON, W. H., AND J. T. SKINNER 1931 Distribution of manganese in foods. *J. Nutrition*, vol. 4, p. 419.
- REMINGTON, R. E., AND H. E. SHIVER 1930 Iron, copper and manganese content of some common vegetable foods. *Assoc. Official Agr. Chem.*, vol. 13, p. 129.
- SKINNER, J. T., W. H. PETERSON, AND H. STEENBOCK 1931 The manganese metabolism of the rat. *J. Biol. Chem.*, vol. 90, p. 65.

# UTILIZATION OF MEAT BY HUMAN SUBJECTS <sup>1</sup>

## I. THE UTILIZATION OF THE NITROGEN AND PHOSPHORUS OF LOIN AND HEEL CUTS OF BEEF

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For those of limited means it is customary to urge the consumption of the less tender and consequently less expensive beef cuts. Since a question has arisen concerning their relative merits as sources of nitrogen in the diet (Mitchell, '27), a balance study was made to determine the efficiency of the nitrogen from loin and heel of beef, representing a most and a least tender cut. The utilization of the phosphorus from the two meats was also studied and their collagen and elastin content determined. The experiment was divided into two parts, each preceded by a 4-day preliminary period and including two 4-day experimental periods. In the first part, the beef loin was used and in the second part, the 'heel.'

The meat was of known origin and good grade. Equal weights of loin and heel were taken from the same carcass so any observed differences in usage could be attributed to cut rather than to grade. The meats were freed from visible fat, coarsely ground once, and each thoroughly mixed. The daily supply for each subject was weighed in two portions, frozen quickly, and kept frozen until used.

The subjects were three healthy young women of 70 kg. each, using from 34.5 to 41.0 Calories per kilogram. Their diet (table 1) was base-forming. Breakfast consisted of a nitrogen-free bread, filtered butter fat and a fruit drink.

<sup>1</sup> Contribution no. 32, Department of Home Economics.

These same foods were eaten with the meat at lunch and dinner and two subjects also ate a fondant-like candy. Distilled water was used ad libitum. Ferric citrate and calcium carbonate were added to bring the calcium and iron up to accepted standards (Sherman, '32).

The diet furnished from 87.5 to 87.8 per cent of the daily protein requirement of 44.4 gm. per 70 kg. (Sherman, '32),

TABLE 1  
*Daily food intake in loin of beef period<sup>1</sup>*

SUBJECT	FOOD	WEIGHT	PROTEIN	PHOSPHORUS	CALORIES
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
A	Meat	165.0	36.6	0.341	190
	Bread <sup>2</sup>				972
	Butterfat, filtered	27.0			243
	Beverage <sup>3</sup>	838.2	2.3	0.093	1099
	Candy <sup>4</sup>				365
	CaCO <sub>3</sub>	1.375			
	FeC <sub>2</sub> H <sub>3</sub> O <sub>7</sub>	0.041			
	NaCl	Ad libitum			
	Total		38.9	0.434	2869
B	Same as A, omitting candy and 10 gm. butter fat; 2414 calories.				
C	Same as A, omitting 10 gm. butter fat; 2779 calories.				

<sup>1</sup> In the heel of beef period the protein totaled 38.5 gm. and the phosphorus 0.432 gm. per day.

<sup>2</sup> Bread.

	<i>gm.</i>
Arrowroot starch	120
Lactose	40
Dextrin-maltose	15
Baking powder, tartrate	3
Sodium chloride	3
Agar-agar	4
Butter fat, filtered	32
Water, distilled	80

<sup>3</sup> Beverage.

	<i>gm.</i>
Grape juice	600.0
Lemon juice	128.2
Lactose	85.0
Sucrose	75.0

<sup>4</sup> Candy.

	<i>gm.</i>
Sucrose	75
Arrowroot starch	5
Butter fat, filtered	5
Water, distilled	60

the meat supplying 94.2 to 94.5 per cent of the total nitrogen and 78.2 to 78.9 per cent of the total phosphorus. The latter provided only 49.1 to 49.3 per cent of the daily phosphorus requirement of 0.88 gm. per 70 kg. of body weight (Sherman, '32).

The customary precautions were taken for care of samples. Urine was collected quantitatively for each 24-hour day and feces for each 4-day period. Total nitrogen was determined

by the Kjeldahl-Gunning method, phosphorus by the volumetric procedure of the Association of Official Agricultural Chemists, and collagen and elastin by revised directions from the Illinois Agricultural Experiment Station.

TABLE 2  
*Average daily nitrogen and phosphorus balances*

SUBJECT	CUT OF MEAT	AVERAGE DAILY INTAKE		AVERAGE DAILY OUTPUT			BALANCE
		Meat	Total	Urine	Feces	Total	
Nitrogen <sup>1</sup>							
		gm.	gm.	gm.	gm.	gm.	gm.
A (70 kg.)	Loin <sup>2</sup>	5.86	6.22	4.24	1.31	5.55	+ 0.67 ± 0.10
	Heel <sup>3</sup>	5.82	6.16	4.95	1.41	6.36	— 0.20 ± 0.13
B (70 kg.)	Loin	5.86	6.22	5.16	0.70	5.86	+ 0.36 ± 0.12
	Heel	5.82	6.16	5.19	0.83	6.02	+ 0.14 ± 0.11
C (70 kg.)	Loin	5.86	6.22	4.00	1.47	5.47	+ 0.75 ± 0.20
	Heel	5.82	6.16	4.26	1.68	5.94	+ 0.22 ± 0.21
All subjects	Loin	5.86	6.22	4.47	1.16	5.63	+ 0.59 ± 0.08
	Heel	5.82	6.16	4.80	1.31	6.11	+ 0.05 ± 0.09
Phosphorus <sup>1</sup>							
A (70 kg.)	Loin	0.341	0.434	0.320	0.944	1.264	— 0.830 ± 0.154
	Heel	0.338	0.432	0.402	1.140	1.542	— 1.110 ± 0.190
B (70 kg.)	Loin	0.341	0.434	0.349	0.712	1.061	— 0.627 ± 0.115
	Heel	0.338	0.432	0.352	0.857	1.209	— 0.777 ± 0.566
C (70 kg.)	Loin	0.341	0.434	0.279	1.155	1.434	— 1.000 ± 0.294
	Heel	0.338	0.432	0.243	1.492	1.735	— 1.303 ± 0.305
All subjects	Loin	0.341	0.434	0.316	0.937	1.253	— 0.819 ± 0.072
	Heel	0.338	0.432	0.332	1.163	1.495	— 1.063 ± 0.104

<sup>1</sup> Average of 8 experimental days.

<sup>2</sup> Collagen furnished 6 per cent of total nitrogen; elastin, 1.1 per cent.

<sup>3</sup> Collagen furnished 12.2 per cent of total nitrogen; elastin, 0.9 per cent.

With a daily intake of 0.089 gm. nitrogen per kilogram, the average daily retention for all subjects was + 0.59 ± 0.08 gm. for the entire 8 days of the beef loin period (table 2). This indicated good usage of loin nitrogen. Two subjects were in negative balance on each of 2 days, but only once for each was this appreciable in amount. With heel of beef supplying



0.088 gm. of nitrogen per kilogram daily, all subjects were close to nitrogen equilibrium, one being negative and the other two slightly positive. The average daily retention of nitrogen for all subjects on this cut was  $+0.05 \pm 0.09$  gm., although on half of the experimental days the balances were negative.

Digestibility varied more with the subjects than with the meats. The average coefficient of digestibility for the nitrogen of the loin of beef for the various subjects ranged from 69.5 to 91.3 per cent and averaged 81.3 per cent for all subjects. In the heel period it ranged from 63.8 to 87.7 per cent with an average of 78.8. Urinary nitrogen amounted to 0.064 gm. per kilogram per day in the loin period and 0.069 gm. for the heel period, again suggesting slightly better usage of the nitrogen from the tender cut.

Collagen nitrogen, calculated as percentage of total nitrogen, was twice as high in the heel as in the loin (table 2) while the elastin nitrogen was nearly the same. The difference in the average for the nitrogen balances for the two cuts which is 4.5 times its P. E. ( $\frac{0.54}{0.12} = 4.5$ ) may be regarded as significant and possibly may be explained by the difference in the original collagen content of the meats and the degree to which it was converted into gelatin in cooking. It cannot be attributed to elastin, since the slight difference in this case was in favor of the less tender meat. Variations in individual subjects are also apparent in these results.

A negative phosphorus balance was obtained in all cases, as was expected with a daily intake of approximately 6 mg. per kilogram of body weight or half the actual requirement. The individual phosphorus balances for the beef loin period ranged from  $-0.457$  to  $-1.365$  gm. per day and averaged  $-0.819 \pm 0.072$  gm. for all subjects. The results were less favorable for the heel of beef with practically the same intake. The daily average phosphorus balance for this period was  $-1.063 \pm 0.104$  gm. and varied from  $-0.693$  to  $-1.751$  gm. for the different subjects. Fecal phosphorus for the heel of beef periods was slightly higher than for the loin paralleling a somewhat lower coefficient of digestibility for nitrogen and

suggesting greater phosphorus losses in digestion. Phosphorus balances were more negative for all subjects in the second period for each cut of meat indicating inability to adjust to so low an intake. The difference in the average phosphorus balances for all subjects for the two meats of  $-0.244 \pm 0.126$  gm. is not significant and suggests less difference in quality of phosphorus than was noted for nitrogen.

#### CONCLUSION

When heel of beef is substituted for loin in the diet as a source of nitrogen and phosphorus, the amount should be increased slightly, since these elements appear to be somewhat better used from the more tender cut. The differences for nitrogen appear to be greater than for phosphorus.

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#### LITERATURE CITED

- MITCHELL, H. H. 1927 The protein values of foods in nutrition. *J. Home Econ.*, vol. 19, p. 122.
- SHERMAN, H. C. 1932 *Chemistry of food and nutrition*, 4th ed. The Macmillan Co., New York.



# THE SPECIFIC DYNAMIC EFFECTS OF PROTEIN, FAT, AND CARBOHYDRATE AS DETERMINED WITH THE ALBINO RAT AT DIFFERENT PLANES OF NUTRITION<sup>1</sup>

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## INTRODUCTION

In a previous publication (Forbes, Kriss and Miller, '34) confirmatory evidence was presented to the effect that the relationship between the plane of nutrition and the heat production of the albino rat, from the fasting level to the level of one and a half times maintenance, is represented not by a straight line, but by a curve of increasing steepness of upward slope. This phenomenon may be interpreted to signify either, 1) that the specific dynamic effects of nutrients increase with the rise in plane of nutrition or, 2) that the current method of computation of the specific dynamic effects of nutrients, which relates the nutrients ingested to the resultant increase in metabolism over the basal level, needs revision.

The first suggestion of the reasonableness of the latter point of view came from Rubner as long ago as 1902, when he made the observation that the administration of phlorhizin to a fasting dog caused an increase in the heat production coincident with an increase in body protein katabolism, as compared with the normal fasting values.

<sup>1</sup>Publication authorized by the director of the Pennsylvania Agricultural Experiment Station, March 16, 1934, as technical paper no. 644.

Rubner considered the results of this experiment as a demonstration of the existence of a 'specific dynamic effect' of body protein, when katabolized, similar to that of food protein. This view seems to have been accepted, in part at least, by Lusk, for in reviewing this work ('28) he stated that "this increase (in heat production) Rubner rightly attributed to the specific dynamic action of increased (body) protein metabolism."

In accord with this conception Williams, Riche and Lusk ('12) proposed a method of calculating the specific dynamic effect of protein which accounted for that of the body protein katabolized during fasting by relating the extra protein metabolized, instead of the total protein ingested, to the increase in the heat production over the basal level. This principle, however, has been disregarded by many workers in the field.

In a critical analysis of published data, largely those of Lusk and his pupils, Borsook and Winegarden ('31 a) observed that the specific dynamic effect of protein parallels the course of nitrogen excretion. These authors have shown that when the increase in heat production following the ingestion of proteins or amino acids is compared with the increase, over the basal level of excretion, of urinary nitrogen, a close correlation is obtained, while failure to account for the basal nitrogen excretion, or for the incompleteness of metabolism of the nitrogen ingested, might be the cause of anomalous results. These observations of Borsook and Winegarden, which find support in the work of Wilhelmj and Bollman ('28), Wilhelmj and Mann ('30), Wilhelmj, Bollman and Mann ('31), Wishart ('28) and of Wilhelmj ('34) recall the early idea of Rubner ('02) that when body tissue is katabolized it exerts a specific dynamic effect, which is, of course, not the same as its heat of oxidation.

Rubner's contention that body protein and ingested protein have the same specific dynamic effect and that the higher basal metabolism in the phlorhizinized dog is the result of the specific dynamic effect of the increased destruction of body

protein finds support in the observations by Chambers and Lusk ('30) that the heat production rises to the same height after the administration of meat to the normal dog and afterward to the same dog when phlorhizinized. Additional evidence in favor of this explanation is found in the experiments by Dann, Chambers and Lusk ('31) with thyroidectomized dogs.

In seeking an explanation for the curvilinear relationship between the heat production and the plane of nutrition, Forbes, Braman and Kriss ('28, '30) and later, Forbes and Kriss ('32) concluded—in harmony with the ideas expressed above—that the fasting heat production is greater than the hypothetical minimum energy requirement by the amount of the waste heat of the body nutrients katabolized during fast; that the heat production between the fasting and the maintenance levels is affected by this factor in progressively diminishing degrees; and that the heat production at or above the maintenance level is entirely unaffected by this factor.

The purpose of the present investigation was to throw further light on these conclusions, and to explain the differences observed in experiments with cattle, between the dynamic effects of food above and below maintenance.

#### EXPERIMENTAL PROCEDURE

The subjects employed in this investigation were albino rats weighing about 100 gm. at the beginning of the experiments. Several series of respiration experiments were conducted as follows:

1. For the determination of the specific dynamic effects of protein, carbohydrate and fat, at supermaintenance planes nine female rats were used, all of which were subjected to the following dietary treatments in the different experimental periods (table 1): 1. Maintenance basal ration consisting of 5.5 gm. per day of a commercial mixed calf meal and estimated to maintain the animals in approximate energy equilibrium. 2. Basal ration (as in 1) plus 2 gm. per day of casein (Harris). 3. Basal ration (as in 1) plus 2.2 gm. per day of

cornstarch. 4. Basal ration (as in 1) plus 1 gm. of olive oil per day. The quantities of casein, starch and oil added to the basal ration were approximately equicaloric, and together with the basal ration constituted an excess of energy over the maintenance requirement equivalent to approximately one-half of the latter.

The calf meal used as the basal ration was of the same composition as given in the preceding paper.

TABLE 1

*Schedule of supermaintenance respiration experiments, and body weights*

RAT NO.	BASAL RATION 5.5 GM.		BASAL RATION PLUS 2 GM. CASEIN		BASAL RATION PLUS 2.2 GM. STARCH		BASAL RATION PLUS 1 GM. OLIVE OIL	
	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
	1931	Gm.	1931	Gm.	1931	Gm.	1931	Gm.
1199	Nov. 16	108	Nov. 23	123	Nov. 30	126	Dec. 7	132
1301	Nov. 18	106	Nov. 25	121	Dec. 2	127	Dec. 9	132
1300	Nov. 20	98	Dec. 3	119	Dec. 10	124	Dec. 17	126
	1932		1932		1932		1932	
387	Jan. 4	104	Jan. 14	124	Jan. 20	128	Jan. 28	138
386	Jan. 6	99	Jan. 11	113	Jan. 18	122	Jan. 25	132
388	Jan. 7	98	Jan. 13	111	Jan. 21	126	Jan. 27	132
385	Jan. 8	91	Jan. 15	106	Jan. 22	114	Jan. 29	
451	Oct. 3	98	Oct. 11	104	Oct. 19	115	Oct. 24	124
453	Oct. 3	99	Oct. 11	106	Oct. 18	117	Oct. 24	125

2. For the determination of the specific dynamic effects of protein, carbohydrate and fat at the maintenance level six female and eight male rats were used. All of these animals were subjected to a basal metabolism test. Five of the female rats and five of the male rats were subjected, in different periods, to an exclusive protein diet consisting of 3.8 gm. per day of casein (Harris); four of the female rats and five of the male rats were subjected to an exclusive carbohydrate diet consisting of 3.92 gm. of cornstarch daily; and three of the female and four of the male rats were subjected to an exclusive fat diet consisting of 1.56 gm. daily of olive oil. The quantities of casein, starch and oil fed in this series

were estimated to maintain the animals in approximate energy equilibrium. As will be seen from the schedule of experimentation (table 2), the majority of the animals were subjected, at different times, to two experimental dietary treatments in addition to fasting. In only one case were all four experimental treatments imposed on the same animal (rat no. 34). Although no obvious ill effects on the animal were observed in this case, the number of experimental treatments imposed on the same animal was limited to three in all other

TABLE 2

*Schedule of maintenance respiration experiments, and body weights*

RAT NO.	SEX	CASEIN (3.8 GM. PER DAY)		STARCH (3.92 GM. PER DAY)		OLIVE OIL (1.56 GM. PER DAY)		FASTING	
		Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
		1932	Gm.	1932	Gm.	1932	Gm.	1932	Gm.
61	F	Mar. 14	111	Mar. 21	108	Mar. 25	84	Mar. 28	106
62	F	Mar. 16	104	Mar. 23	102			Mar. 30	103
64	F	Mar. 18	82			Mar. 25	84	Apr. 1	90
63	F					Mar. 24	90	Mar. 31	97
58	M			Apr. 13	94	Apr. 19	87		
57	M			Apr. 14	92	Apr. 20	88	Apr. 25	97
59	M			Apr. 15	91			Apr. 26	97
56	M			Apr. 18	94			Apr. 21	97
34	F	Aug. 8	96	Aug. 15	95	Aug. 22	87	Aug. 29	93
38	M	Aug. 17	101	Aug. 26	94			Sept. 1	98
35	M	Aug. 18	97	Aug. 25	94			Sept. 7	92
36	F	Aug. 19	111	Aug. 30	108			Sept. 7	114
31	M	Sept. 2	98			Sept. 9	90	Sept. 13	94
37	M	Sept. 6	103			Sept. 14	92	Sept. 19	100
33	M	Sept. 8	100			Sept. 15	93	Sept. 20	100

cases in order to avoid any possible ill effects of prolonged confinement to the restricted and incomplete diets employed.

All of the diets used were analyzed for energy, and the basal ration and casein were also analyzed for nitrogen. In special metabolism tests with control animals the urinary nitrogen excretion was determined for each diet; and the metabolizability of the energy of the basal diet (calf meal) and of the casein was determined in special balance experiments.



The rats were fed individually, twice daily, and were kept on the experimental diet for at least 3 days prior to the respiration experiments. During the intermediate or transition periods the rats received a maintenance ration of the basal diet. The respiration measurements began as soon as the morning feed was consumed, and, in the case of fasting, they began 24 hours after the last portion of feed was given. The respiration experiments were usually conducted for 7 hours, during which the hourly  $\text{CO}_2$  production and the total respiratory quotients were determined, by means of the apparatus and general technic described in the preceding paper (Forbes, Kriss and Miller, '34).

The heat production was computed on the basis of, 1) the hourly  $\text{CO}_2$  production (omitting the weights of  $\text{CO}_2$  for the first 2 hours, for reasons explained in the preceding paper); 2) the protein metabolism, as measured by the urinary nitrogen excretion of control animals and, 3) the non-protein respiratory quotients. As in the previous investigation all the data for heat production were computed to a basis of 100 gm. of empty body weight, the empty weights of the experimental animals being calculated on the basis of the data for alimentary fill of rats, under similar dietary treatments reported elsewhere by Miller and Kriss ('34).

#### DISCUSSION OF RESULTS

##### *Heat production and respiratory quotients*

The hourly heat production of the rats, under the different dietary conditions, and the corresponding respiratory quotients and statistical constants, are presented in tables 3 to 5, inclusive. Table 3 contains the data for the rats which received the basal maintenance ration alone, and for those which received this ration supplemented with casein, starch or olive oil. Tables 4 and 5 contain the corresponding data for the male and female rats, respectively, while fasting, and while receiving the maintenance diets of casein, starch, or olive oil alone. The data for the hourly heat production of the individual rats are all presented in consecutive order.

TABLE 3

Hourly heat production of female rats, per 100 gm. of empty body weight, and respiratory quotients, as influenced by the addition of casein, starch, and olive oil to a basal maintenance ration<sup>1</sup>

RAT NO.	BASAL RATION (5.5 GM. CALF MEAL)			BASAL RATION PLUS 2 GM. CASEIN			BASAL RATION PLUS 2.2 GM. STARCH			BASAL RATION PLUS 1 GM. OLIVE OIL		
	Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.	
		Total	Non-protein		Total	Non-protein		Total	Non-protein		Total	Non-protein
1199	649			754			791			710		
	651			678			771			754		
	651	0.93	0.96	678	0.97	1.18	771	0.97	1.01	754	0.91	0.93
	607			709			807			738		
	689			903						822		
1301	654			761			737			695		
	639			761			737			704		
	639	0.96	1.00	827	0.97	1.16	618	0.98	1.02	704	0.92	0.95
	654			823			836			848		
	677			778			617			881		
1300	672			779			695			684		
	687			732			708			700		
	687	0.94	0.97	732	0.93	1.10	720	1.05	1.11	700	0.88	0.90
	756			679						782		
	761			691						715		
387	642			766			679			635		
	645			877			679			800		
	645	0.95	0.99	877	0.95 <sup>a</sup>	1.12	651	1.03	1.09	800	0.89	0.91
	585			772			696			719		
	721			771						718		
386	635			786			783			740		
	662			787			713			762		
	662	0.95	0.99	787	0.97	1.17	713	1.06	1.12	762	0.91	0.93
	702			801			779			706		
	727			781						741		
388	654			780			886			795		
	672			770			886			792		
	672	0.96	1.00	780	0.95	1.13	882	1.01	1.05	792	0.87	0.88
	620						877			828		
	677									833		
385	603			779			833			581		
	604			779			705			651		
	603	0.96	1.00	817	0.96	1.15	705	1.00	1.04	652	0.88	0.90
	612			786						763		
										699		
451	669			771			743			686		
	669			748			743			712		
	622	0.95	0.99	748	0.93	1.10	712	1.05	1.11	712	0.92	0.95
	638			771			658			622		
				695								
453	741			784			681			655		
	741			770			562			641		
	684	0.91	0.94	770	0.95	1.15	660	1.08	1.15	641	0.93	0.96
	639			787						624		
				705								
Mean	662 ± 4.4	0.95	0.98	769 ± 5.1	0.95	1.14	739 ± 8.7	1.02	1.08	727 ± 6.9	0.90	0.92
Standard deviation of means	6.5 ± 0.49			7.6 ± 0.56			12.9 ± 1.07			10.2 ± 0.75		
Standard deviation	42.1 ± 3.18			49.2 ± 3.63			75.2 ± 6.24			66.9 ± 4.92		

<sup>1</sup> The quantities of casein, starch and olive oil were not exactly equicaloric. The influence on the average daily heat production of these nutrients on an equicaloric basis is shown in table 5.

<sup>a</sup> Assumed.

Attention is called to the fact that the rats were unusually quiet in the chamber after it was placed in the water bath under the electric light. The animals were, however, unusually restless while they were being weighed in the closed jar, thus causing an excessive accumulation of  $\text{CO}_2$ . For this reason, principally, the  $\text{CO}_2$  measurement for the first hour of the experimental period was always excessively high, and with the rate of ventilation (about 35 liters per hour) maintained in the present series of experiments a partial carrying over of this effect into the second hour was also evident in most cases. On this account, the  $\text{CO}_2$  measurements during the first 2 hours of the experimental periods were omitted from the computations of the heat production.

This procedure is especially justified by the fact that our data do not show any rise in heat production during the first few hours after feeding such as is usually observed during realimentation following a period of fasting. In other words, under our conditions of experimentation the heat production of the animals, unaffected by irregularities in activity, is not curvilinear, but is practically on a level. In a subsequent experiment in which the ventilation was at a higher rate than that maintained in the present series we have actually observed that within less than an hour after feeding (a super-maintenance diet) the heat production was brought to a level which was practically maintained throughout the experimental period of 12 hours.

Feeding the animals twice a day did not allow them to be reduced to a fasting state during the intervals between feeding, and no very large fluctuations in the respiratory quotients during the experimental periods could, under the conditions, be expected. Hence, any errors in the computation of the individual hourly heat production by the use of the factors for the average respiratory quotient obtained during the experimental period should be relatively small.

Because the experimental periods were fairly long, the average respiratory quotients possess a high degree of accuracy. This is evidenced by the uniformity in the respiratory quotients for the individual animals on the same diets.

The mean hourly heat production (table 4) for the fasting female rats (518 calories) was almost identical with the corresponding mean (table 5) for the male rats (520 calories). In the previous study (Forbes, Kriss and Miller, '34) the average hourly heat production for a group of eight male

TABLE 4

*Hourly heat production of female rats, per 100 gm. of empty body weight, and respiratory quotients during fast and when receiving equicaloric quantities of casein, starch or olive oil exclusively*

RAT NO.	FASTING			CASEIN (3.8 G.M. PER DAY)			STARCH (3.92 GM. PER DAY)			OLIVE OIL (1.56 GM. PER DAY)		
	Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.	
		Total	Non-protein		Total	Non-protein		Total	Non-protein		Total	Non-protein
61	499			736			601					
	610			672			560					
	611	0.69	0.65	672	0.82		560	1.11	1.13			
	577						504					
	522						561					
62	454			681			620					
	500			685			620					
	500	0.74	0.72	769	0.82		559	1.07	1.09			
	457			755			591					
	506						504					
64	470			719						546		
	516			742						561		
	516	0.75	0.73	742	0.83					561	0.70	0.68
	601			729						568		
	656			778						606		
63	502									510		
	455									533		
	455	0.74	0.72							533	0.72	0.71
	484									551		
	515									604		
34	544			639			617			621		
	546			581			589			540		
	546	0.73	0.71	581	0.82 <sup>1</sup>		588	1.05	1.07	505	0.71	0.70
	574			584			660			505		
				621			612			557		
36	602			653			594					
	421			659			539					
	445	0.73	0.70	648	0.82		552	0.99	1.00			
	445			648			552					
	496			649			546					
Mean	518 ± 7.4	0.73	0.71	679 ± 8.5	0.82		576 ± 5.9	1.06	1.07	553 ± 6.0	0.71	0.70
Standard deviation of means	10.9 ± 0.98			12.6 ± 1.31			8.7 ± 0.95			8.9 ± 1.13		
Standard deviation	58.7 ± 5.28			59.1 ± 6.14			38.9 ± 4.25			34.5 ± 4.38		

<sup>1</sup> Assumed.

TABLE 5

*Hourly heat production of male rats, per 100 gm. of empty body weight, and respiratory quotients during fast, and when receiving equicaloric quantities of casein, starch or olive oil exclusively*

RAT NO.	FASTING			CASEIN (3.8 G.M. PER DAY)			STARCH (3.92 GM. PER DAY)			OLIVE OIL (1.56 GM. PER DAY)		
	Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.	
		Total	Non-protein		Total	Non-protein		Total	Non-protein		Total	Non-protein
57	519						643			562		
	509						664			623		
	509	0.75	0.73				664	1.10	1.12	623	0.73	0.72
	553						615			599		
	551						596			599		
59	564						606					
	514						615					
	514	0.75	0.73				615	1.11	1.13			
	520						590					
	591						605					
56	507						614					
	531						639					
	532	0.76	0.75				639	1.07	1.09			
	500						640					
	549						638					
38	524			763			545					
	549			723			568					
	538	0.72	0.69	723	0.82		584	1.11	1.13			
	538			741			584					
	482			713			622					
35	591			702			572					
	564			702			613					
	562	0.75	0.73	634	0.84		635	1.07	1.09			
	562			672			635					
	615						597					
33	470			645						492		
	472			625						473		
	456	0.72	0.69	640	0.81					479	0.69	0.67
	456			640						480		
	491			632						490		
31	555			708						599		
	463			662						501		
	463	0.74	0.72	628	0.78					591	0.70	0.68
	451			629						591		
				640						598		
37	517			594						530		
	485			619						505		
	507	0.74	0.72	648	0.83					543	0.70	0.68
	506			648						543		
	491			622						502		
Mean	520 ± 4.3	0.74	0.72	665 ± 6.1	0.82		614 ± 3.8	1.09	1.11	546 ± 7.8	0.71	0.69
Standard deviation of means	6.4 ± 0.50			9.1 ± 0.90			5.7 ± 0.55			11.6 ± 1.27		
Standard deviation	40.0 ± 3.12			44.6 ± 4.41			28.5 ± 2.75			51.9 ± 5.68		

rats was found to be 530 calories. While the differences are in favor of the male rats, they are too small to be considered significant, and the agreement between the data representing the two different groups of male rats may be taken as an indication that the conditions of experimentation were satisfactorily controlled.

Both the total and the non-protein respiratory quotients are given in the tables. The computation of the non-protein respiratory quotients, and of the protein metabolism, in all experimental periods except those in which casein was fed, was based on Loewy's ('11) factors, which are generally used. These factors are:

- 1 mg. of urinary nitrogen is equivalent to 4.75 cc. of CO<sub>2</sub>,
- 1 mg. of urinary nitrogen is equivalent to 5.94 cc. O<sub>2</sub>,
- 1 mg. of urinary nitrogen is equivalent to 26.51 calories

The computation of the protein metabolism in the casein experiments was based on factors experimentally determined by Kriss and Miller ('34) for casein. These are:

- 1 mg. of urinary nitrogen is equivalent to 5.47 cc. CO<sub>2</sub>,
- 1 mg. of urinary nitrogen is equivalent to 6.67 cc. O<sub>2</sub>,
- 1 mg. of urinary nitrogen is equivalent to 30.59 calories
- 1 liter of respiratory O<sub>2</sub> is equivalent to 4586 calories

While the protein metabolism was taken into consideration in all of the calculations of the heat production in these experiments, a study of the experimental data revealed that the added accuracy gained by so doing was of minor degree. A comparison of the average hourly heat production as computed from the total and from the non-protein respiratory quotients gave differences ranging from 0 to 3.7 per cent, and, with the exception of the starch experiments, the figures computed from the non-protein respiratory quotient were the lower.

The non-protein respiratory quotients in the fasting experiments averaged 0.71 (table 4) and 0.72 (table 5). These data indicate that 24 hours after the last feeding the rats had reached a status of essentially true fast. The non-protein respiratory quotients in the olive oil periods were slightly

lower than those obtained during fast, suggesting that the oil feeding probably resulted in slight ketosis.

Non-protein respiratory quotients somewhat higher than unity were obtained in the periods in which starch or casein was added to the basal ration (table 3), as well as in the period in which starch was fed exclusively (tables 4 and 5). It appears that 3.92 gm. of starch per day was slightly more than sufficient to supply the energy need for the maintenance of the rats under the experimental conditions, and that the addition of 2 gm. of casein or of 2.2 gm. of starch to the basal ration of calf meal rendered some carbohydrate available for conversion into fat.

In the periods in which casein was fed exclusively (tables 4 and 5) the total respiratory quotients average 0.82, with only slight variation. These respiratory quotients, representing mainly protein metabolism, are identical with the respiratory quotient found by Kriss and Miller ('34) to represent the metabolism of pure casein. The average non-protein respiratory quotient may therefore be taken to be essentially the same as the total respiratory quotient. Since the proportions of non-protein material metabolized in these periods were relatively very small, the non-protein respiratory quotients for the individual rats were considered, in these cases, to be without special significance, and were therefore omitted from the tables.

*Percentage contributions of heat from protein, fat and carbohydrate and the sparing of body nutrients by food nutrients*

Preliminary to calculations of the specific dynamic effects of the various nutrients studied, calculations were made (table 6) of the proportions of the total heat production derived from protein katabolism, carbohydrate katabolism, fat katabolism, and from the synthesis of fat from carbohydrates, under the different dietary conditions. The derivation of these data was based on the urinary nitrogen excretion of control animals, the respiratory quotients, and the total heat produc-

tion. These data are of especial significance in showing the sparing effect of one nutrient upon the other, and have an important bearing on the determination of the specific dynamic effects of these nutrients.

Thus, it will be observed that while during fast the protein metabolism contributed 26.0 per cent to the heat production, this percentage dropped to 5.6 in the case of starch feeding.

TABLE 6

*Urinary nitrogen excretion and percentage contributions of heat from protein, fat and carbohydrates to the total heat production*

DAILY FOOD INTAKE	NITROGEN METABOLISM TESTS			AVERAGE PERCENTAGE OF TOTAL HEAT PRODUCED BY			
	Number of animals	Urine collection periods, days	Average milli-grams urinary nitrogen per day <sup>1</sup>	Protein katabolism	Carbohydrate katabolism	Fat katabolism	Fat synthesis
Basal ration (5.5 gm. calf meal)	7	2	112 ± 3.9	18.7	76.1	5.2	....
Basal ration plus 2 gm. casein	6	2	333 ± 5.7	55.2	43.5	....	1.3 <sup>2</sup>
Basal ration plus 2.2 gm. starch	4	2	108 ± 4.2	16.1	82.6	....	1.3 <sup>2</sup>
Basal ration plus 1 gm. olive oil	4	2	102 ± 3.6	15.5	62.6	21.9	....
Fasting	10	1	122 ± 4.2	26.0	....	74.0	....
3.8 gm. casein	5	3	508 ± 6.2	96.4	1.5	2.1	....
3.92 gm. starch	4	2	30 ± 1.2	5.6	92.6	....	1.8 <sup>2</sup>
1.56 gm. olive oil	4	2	70 ± 2.5	14.1	....	85.9	....

<sup>1</sup> Per 100 gm. empty body weight.

<sup>2</sup> When the non-protein respiratory quotient is above 1.00, for each 5.047 calories non-protein katabolized, 1.09 × (respiratory quotient — 1) calories are synthesized (Lusk, G., Science of Nutrition, 4th ed., p. 397).

In absolute amounts the daily protein metabolism was reduced from 3234 calories during fast to 795 calories when 3.92 gm. of starch were fed; or, in terms of urinary nitrogen, the reduction was from 122 mg. during fast to 30 mg. in the starch feeding periods. In the later case, therefore, the protein metabolism dropped to about one-fourth of the fasting value. Similarly, when 1.56 gm. of olive oil was fed to the



rats, the protein metabolized (70 mg. urinary nitrogen) was only slightly more than one-half of the fasting protein metabolism, the contribution to the total heat production being 14.1 per cent.

If there is a dynamic effect of body nutrients katabolized, therefore, the apparent specific dynamic effects of carbohydrate or of fat, as determined under any conditions such that they spare the katabolism of body nutrients, must be values of mixed significance.

There is evidence in the literature that the elaboration and excretion of urinary nitrogen is attended by a considerable expenditure of energy. This subject has been adequately discussed by Borsook and Winegarden ('31 a, b, c) and by Borsook and Keighley ('33 a, b). If this is true, the ideal condition for determining the specific dynamic effect of a non-protein nutrient, involving the comparison of the heat production in two experimental periods, would be that the protein metabolism be the same in the two periods compared. As will be observed, such a condition practically prevails when the comparison is made between a maintenance ration and the same ration plus a non-protein supplement, but does not prevail in any comparison involving the fasting metabolism.

Furthermore, it is not to be expected that the so-called sparing effect of non-protein food nutrients on the body protein metabolism would be the same regardless of the quantity of the substance fed. On the contrary, this effect may be expected to vary greatly with the quantity of such food nutrients.

The foregoing considerations imply that more consistent and presumably more significant values for the specific dynamic effects of carbohydrates and fats might be obtained if the differences in protein metabolism between the periods compared could be taken into account, or if the heat production of the animal maintained in energy and protein equilibrium, instead of the fasting heat production, be made the base value for comparison with higher levels.

The feeding of casein naturally resulted in large increases in protein metabolism. When 3.8 gm. of casein was fed practically all of the absorbed nitrogen (540 mg.) was metabolized and excreted in the urine, contributing 96.4 per cent of the total heat production. In this case all of the urinary nitrogen (508 mg.) is to be considered as representing casein metabolism.

The feeding of casein, therefore, in quantity sufficient to supply the entire maintenance requirement of energy and of protein, completely protected the body protein from katabolism. This sparing effect of protein feeding, or the replacement of body protein by food protein, should be taken into consideration in determinations of the specific dynamic effects of proteins.

Thus, when a comparison is made, as is usual in determinations of the specific dynamic effects, between the heat production of fasting and the heat production of the protein feeding, this comparison, in relation to the amount of casein under discussion, is as between zero casein intake and 3.8 gm. casein intake, but in relation to protein metabolism, it is not as between zero protein metabolism and the metabolism of 3.8 gm. casein, because during fast some protein (from the body) is metabolized, which involves work by the kidneys, and an expenditure of energy aside from the heat of oxidation of protein per se.

Above the maintenance requirement, as when comparing the basal ration (5.5 gm. calf meal) with the same ration plus any of the supplements, there is, of course, no replacement of body nutrients by food nutrients to consider, since the basal ration alone was adequate to protect the animal from loss of body tissue.

The addition of starch or olive oil to the basal maintenance ration had a relatively slight effect on the amount of protein metabolized. The addition of casein to the basal maintenance ration resulted in an increase in urinary nitrogen (110 mg. per gram of casein), which, in relation to the casein added, is similar in magnitude to the increase observed (102 mg. per

gram of casein) between fasting and 3.8 gm. of casein, but markedly different from the value (134 mg. per gram of casein) which expresses the relationship between the 3.8 gm. of casein and the corresponding total urinary nitrogen excretion.

In the foregoing discussion of the effects of feeding carbohydrate, fat and protein at different planes of nutrition, particularly in the discussion of their sparing effect on body protein, as affecting the determination of the specific dynamic effects, it was assumed that body tissue, when metabolized, gives rise to a specific dynamic effect. A test of this hypothesis is found in the comparison of the specific dynamic effects of casein, starch and olive oil as determined by the different methods discussed below.

*Specific dynamic effects of casein, starch and olive oil at supermaintenance and at maintenance planes of nutrition*

For the purpose of determining the specific dynamic effects of the casein, starch and the olive oil tested, the average hourly heat production of the rats under the various dietary treatments, given in tables 3, 4 and 5, was first of all computed to a 24-hour basis. This computation is justified by the observation that the heat production is practically on a level, due, in large measure, to the facts that the rats were fed twice daily and that they were established on the respective experimental dietary regimes for several days prior to the respiration measurements. In other words, the several hours' heat measurements are considered, under the experimental conditions, to be fairly representative of the daily heat production.

The specific dynamic effects of casein, starch and olive oil, at supermaintenance planes of feeding (table 7) were computed from a comparison of the average daily heat production of the rats on 5.5 gm. of the basal maintenance ration with their average daily heat production on the same ration plus the respective supplements. In this computation, therefore, the heat production of maintenance, and not of fast, was taken as the base value.

The ration of 5.5 gm. of calf meal was found, in energy and nitrogen balance experiments, to be required for the maintenance of the body weight of a 100-gm. rat under cage conditions, while under the conditions of more restricted activity in the respiration chamber, 5.5 gm. of calf meal was found to supply some excess of energy over the quantity required to maintain energy equilibrium.

The metabolizable energy value of the casein fed in these experiments was also determined from complete balances of energy and nitrogen (Kriss and Miller, '34), while the computation of the metabolizable energy of the starch and of the

TABLE 7

*Specific dynamic effects of casein, starch and olive oil when added to a basal maintenance ration*

BASAL RATION	DAILY SUPPLEMENT	METABO- LIZABLE ENERGY OF SUPPLE- MENT	AVERAGE DAILY HEAT PRODUCTION PER 100 GM. OF EMPTY BODY WEIGHT	INCREASE IN HEAT PRODU- TION CAUSED BY SUPPLEMENT	
				Total	Per 100 Cals. of metabolizable energy ingested
5.5 gm.		<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
5.5 gm.	2 gm. casein	8166	15,888	2568	31.4
5.5 gm.	2.2 gm. starch	8228	17,736	1848	22.5
5.5 gm.	1 gm. olive oil	9426	17,448	1560	16.5

olive oil was based on the assumption that these substances are completely digested. This assumption is justified mainly by the chemical nature of these substances, sustained by the fact that when either was fed exclusively, to the control groups used in the nitrogen metabolism experiments, no feces, or negligible quantities of feces, were dropped during the observational periods.

The increase in heat production caused by the addition of 2 gm. of casein to the basal maintenance ration (table 7) was 2568 calories. This heat increment is 31.4 per cent of the metabolizable energy of the ingested casein.

The addition of 2.2 gm. of starch (8228 calories) to the basal maintenance ration caused an increase in the heat production of 1848 calories, or 22.5 per cent of the metabolizable energy of the starch.

The addition of 1 gm. of olive oil (9426 calories) to the basal maintenance ration resulted in a heat increment of 1560 calories, or 16.5 per cent of the metabolizable energy of the oil.

In the light of the foregoing discussion, these increases in heat production may be considered the true specific dynamic effects of these substances. Casein exhibits the highest effect, olive oil shows the lowest, while starch takes an intermediate place. This order of magnitude has been shown generally to

TABLE 8

*Specific dynamic effects of casein, starch and olive oil when each of these was fed exclusively to female and male albino rats*

DAILY FOOD	METABOLIZABLE ENERGY OF DAILY FOOD	AVERAGE DAILY HEAT PRODUCTION PER 100 GM. OF EMPTY BODY WEIGHT		INCREASE IN HEAT PRODUCTION OVER FASTING							
				Total		Per 100 calories of metabolizable energy					
						Uncorrected		Corrected for sparing effect on body protein		Corrected for sparing effect on body protein and fat	
		Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
Fasting	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
.....		12,432	12,480	.....	.....	.....	.....	.....	.....	.....	.....
3.8 gm. casein	15,515	16,296	15,960	3864	3480	24.9	22.4	31.5	28.3	36.5	32.2
3.92 gm. starch	14,661	13,824	14,736	1392	2256	9.5	15.4	14.7	20.1	20.2	24.4
1.56 gm. olive oil	14,705	13,272	13,104	840	624	5.7	4.2	8.7	6.9	14.1	11.2

prevail in comparisons of the specific dynamic effects of proteins, fats and carbohydrates, though the actual values for carbohydrate and fat differ considerably from those given by Lusk ('28).

Turning to a consideration of the determination of the specific dynamic effects of casein, starch and olive oil at maintenance levels of feeding (table 8), using the fasting heat production as the base value, for comparison—it will be observed that the metabolizable energy of the daily amounts of casein, starch or olive oil ingested exceeded the daily fasting

heat production, but approximated fairly closely the quantities of heat produced in the respective feeding periods. In other words, the quantities fed represented approximately the maintenance requirements of energy. It was, of course, impossible without a previous knowledge of the specific dynamic effects of the substances tested, to determine the exact quantities required to provide energy equilibrium.

The specific dynamic effects of the casein, the starch and the olive oil were computed by two procedures, first, the method usually followed by students of this subject, by relating the increase in heat production over the fasting metabolism to the metabolizable energy ingested; and, second, by relating to the metabolizable energy the same heat increment corrected for the sparing of body tissue, which has been mentioned. The results obtained with the male and the female rats are given separately.

The fundamental reasons for the necessity of accounting for the sparing of the body protein by foods, in determination of these heat increments, have been previously discussed, and evidence was cited which justifies the assumption that the specific dynamic effect of body protein broken down is of the same order of magnitude as that of food proteins. On the basis of these considerations and of the data of table 6, the corrections for the sparing of body protein by casein, starch and olive oil, respectively, were made as follows:

In respect to fasting, the 3.8 gm. of casein spared 122 mg. of body nitrogen per day, which represent 3234 calories of metabolizable energy. This quota is subtracted from the metabolizable energy of the casein, in order to determine the increase in metabolizable energy of protein which would be referable to the increase in heat production over the fasting metabolism. The specific dynamic effect of the casein is in this case, therefore, corrected for the sparing of body protein indirectly by relating the observed increase in heat production to the metabolizable energy of the protein ingested diminished by the metabolizable energy of the protein broken down during fast.

The 3.92 gm. of starch and the 1.56 gm. of olive oil spared, in relation to fasting, 92 mg. and 52 mg. of body nitrogen, respectively, which represent 2439 calories and 1379 calories of metabolizable energy, respectively. The specific dynamic equivalents of the protein spared are computed on the basis of the specific dynamic values of casein (corrected as above for the sparing of body protein), and are added to the respective increases in heat production over the fasting metabolism. These corrected heat increments are then related to the metabolizable energy of the nutrients ingested.

All values for the specific dynamic effects are expressed as calories per 100 calories of metabolizable energy.

The specific dynamic values computed for the male and female rats show fair agreement, the values for casein and olive oil being somewhat higher with the female rats than with the male rats, while the reverse is true in the case of starch. Apparently these differences in specific dynamic effect are not closely, if at all, related to the sex. The average values for the two groups are, therefore, considered more significant than the values representing either one of these groups.

The heat increment values of either casein, starch, or olive oil determined with respect to fasting (table 8), and uncorrected for the sparing of body protein, are considerably lower than the corresponding values determined above maintenance (table 7).

All heat increment values determined with respect to fasting, with correction for the sparing of body protein, are considerably higher than the corresponding uncorrected values, and in the case of casein the corrected values (averaging 29.9 per cent) are only slightly lower than the specific dynamic effect (31.4 per cent) of this nutrient determined above maintenance.

The average of the heat increment values of starch, corrected for the sparing of body protein (17.4 per cent), while considerably higher than the average of the uncorrected values (12.5 per cent), is still appreciably lower than the

specific dynamic value of starch (22.5 per cent) as determined above maintenance, although the corrected value for the male rats (20.1 per cent) does not differ greatly from the specific dynamic value of starch as determined above maintenance.

The heat increment value of olive oil as determined below maintenance, with correction for the sparing of body protein (averaging 7.8 per cent), is also found to be considerably lower than the corresponding value of olive oil (16.5 per cent), as determined above maintenance.

In the last two columns of table 8 are given values for the specific dynamic effects of casein, starch and olive oil which have been corrected both for the sparing of body protein and for the sparing of body fat. The latter correction represents an attempt to account for any differences between the amounts of body fat katabolized during fast and during feeding of starch, casein, or olive oil, and rests entirely on hypothetical grounds.

In the fasting periods the metabolism of body fat constituted 74.0 per cent of the total heat production (table 6). In the periods in which starch or olive oil was fed, no body fat was broken down. In the casein periods only 2.1 per cent of the total heat production was derived from body fat. On the assumption that the metabolism of body fat produces a specific dynamic effect like that of food fat, the correction for the sparing of body fat by the various nutrients ingested was made by adding the specific dynamic equivalents of the calories of fat spared (calculated on the basis of the corrected specific dynamic values of olive oil) to the respective increases in heat production over fasting, corrected for the sparing of body protein, and relating the heat increments thus corrected to the metabolizable energy of the corresponding nutrients ingested.

It is of much interest to find that the specific dynamic values of casein, starch and olive oil, corrected for the sparing of body protein and fat, agree reasonably well with the corresponding values of these substances determined (as in table 7) above maintenance.



The writers wish to make it clear that in making the foregoing calculations of the different corrections for the specific dynamic effect of body tissue their primary aim has been to subject the various assumptions made, which in their opinion are reasonable, to experimental verification.

In the opinion of the writers, the results obtained contribute to the confirmation of the conclusion of Forbes, Braman and Kriss ('30), from experiments with cattle, that the fasting heat production includes a factor of waste heat of utilization of body tissue broken down, and to the justification of their conclusion that heat increments of rations determined below maintenance are less than the true energy expense of food utilization by the amount of the waste heat of utilization of body nutrients katabolized.

The present understanding of the writers, therefore, is that specific dynamic effects of nutrients should be measured in accord with the principle of the current method of determination, by Forbes and associates, of heat increments of rations for body increase, with the heat production of a status of nitrogen and energy equilibrium as the base value.

This procedure, the general plan of which has been designated and discussed as the feed-and-plane-difference method, was used in its earliest form, by Armsby, many years ago, in experiments with cattle, but without the present understanding of principles involved, and without a definite base value.

In the further development of this method of study of energy metabolism, the difference in heat increments above and below maintenance was established by Forbes, Fries, Braman and Kriss ('26).

The establishment and explanation of the curve of heat production, by Forbes, Braman and Kriss ('28 and '30) and by Forbes and Kriss ('32), in experiments with cattle, yielded the present understanding of the cause and significance of this difference in heat increments, as relating to entire foodstuffs or rations; while the present paper extends this understanding to its relation to proteins, fats and carbohydrates, as individual food constituents, and also definitely specifies that

the basal maintenance ration must provide for nitrogen as well as energy equilibrium. The principle of this last requirement has been understood, as expressed by Forbes ('29, '32, '33), and has been satisfied in the use of this procedure, but has not been emphasized heretofore in the present connection.

In the preceding paper (Forbes, Kriss and Miller, '34) attention was called to the different attitudes expressed by different investigators toward the curve of heat production in relation to the plane of nutrition; and to the different procedures used for the determination of the energy cost of food utilization at different planes of nutrition.

In this connection reference was made to the facts that Mitchell and Hamilton ('32), Wiegner and Ghoneim ('31), and Brody and Procter ('33) calculated the heat increments and net energy values of rations at different planes of nutrition, including supermaintenance planes, by using the fasting heat production as the base value, and in so doing employed the data of Forbes, Braman and Kriss ('28, '30) in ways implying change of interpretation from that of the authors, with the result that they obtained values of mixed significance.

It is the belief of the writers that the clearing up of this existing confusion of thought in the field of energy metabolism depends upon—and is impossible without—an effective recognition of the fact first brought to light by Rubner and substantiated by the last experimental work of Lusk and by the results of the present study that when body tissue is katabolized it is not employed for energy production at 100 per cent efficiency, but gives rise to a waste heat of utilization, and is therefore characterized by a dynamic effect, in the same sense as is food nutriment.

#### SUMMARY AND CONCLUSIONS

The average specific dynamic effects resulting from the addition, to a basal maintenance ration, of casein, starch and olive oil, respectively, expressed as percentages of the metabolizable energy of these supplements, were 31.4 per cent for

casein, 22.5 per cent for starch and 16.5 per cent for olive oil. These values were considerably greater than the corresponding increases in heat production, over the fasting metabolism, resulting from the exclusive feeding of casein, starch and olive oil, when these heat increments were related to the metabolizable energy ingested.

As a contribution to the establishment of the causes of these differences in heat increment values determinations were made, at the different planes of nutrition, of the percentages of the total heat derived from protein, fat and carbohydrate, and of the sparing of body nutrients, under the different dietary treatments.

Attempts were made to confirm the existence of specific dynamic effects of body protein and fat katabolized by accounting for the sparing effects of starch, olive oil and casein, on the katabolism of body tissue.

All heat increment values determined with respect to fasting, with correction for the sparing of body protein, were found to be considerably higher than the corresponding uncorrected values.

The heat increment values of casein, starch and olive oil, corrected for the sparing of both body protein and fat, were found to agree reasonably well with the heat increment values of these substances determined above maintenance—with the heat production of energy and nitrogen equilibrium as a base value.

These conditions (of determination above maintenance) are believed to be as nearly correct as practicable for measuring the specific dynamic effect of any nutrient.

The results obtained constitute fairly satisfactory confirmation of the conclusion of Rubner that the heat produced by the katabolism of body protein includes a factor of waste heat of utilization; and also justify the conclusion of Forbes, Braman and Kriss that heat increment values of rations determined directly with reference to the fasting heat production (uncorrected for the sparing of body tissue) are lower than the true energy expense of utilization by the amounts of the dynamic effect of body substance spared.

## LITERATURE CITED

- BORSOOK, H., AND M. WINEGARDEN 1931 a On the specific dynamic action of protein. *Proc. Nat. Acad. Sci.*, vol. 17, pp. 75-91.
- 1931 b The energy cost of excretion of urine. *Proc. Nat. Acad. Sci.*, vol. 17, pp. 13-28.
- 1931 c The work of the kidney in the production of urine. *Proc. Nat. Acad. Sci.*, vol. 17, pp. 3-12.
- BORSOOK, H., AND G. KEIGHLEY 1933 a The energy of urea synthesis. *Proc. Nat. Acad. Sci.*, vol. 19, pp. 626-631.
- 1933 b The energy of urea synthesis. II. The effect of varying hydrogen ion concentration with different metabolites. *Proc. Nat. Acad. Sci.*, vol. 19, pp. 720-725.
- BRODY, S., AND C. PROCTER 1933 Influence of the plane of nutrition of the utilizability of feeding stuffs. Review of literature and graphic analyses of published data on the net-energy and specific dynamic action problems. *Missouri Research Bulletin* 193.
- CHAMBERS, WILLIAM H., AND GRAHAM LUSK 1930 Specific dynamic action in the normal and phlorhizinized dog. *J. Biol. Chem.*, vol. 85, p. 611.
- DANN, MARGARET, WILLIAM H. CHAMBERS AND GRAHAM LUSK 1931 The influence of phlorhizin glycosuria on the metabolism of dogs after thyroidectomy. *J. Biol. Chem.*, vol. 94, p. 511.
- FORBES, E. B. 1929 The problem of determination of net energy values of feeding stuffs. *Am. J. Physiol.*, vol. 90, p. 348.
- 1932 Net energy of completely balanced nutriment as a measure of nutritive value of rations and of nutritive requirements of animals. *Proc. Am. Soc. Anim. Production*, Twenty-fifth Annual Meeting, Nov. 25-26, 1932, publ. 1933, pp. 32-40.
- 1933 The law of maximum normal nutritive value. *Science*, vol. 77, pp. 306-307.
- FORBES, E. B., W. W. BRAMAN AND M. KRISS 1928 The energy metabolism of cattle in relation to the plane of nutrition. *J. Agric. Research*, vol. 37, p. 254.
- 1930 Further studies of the energy metabolism of cattle in relation to the plane of nutrition. *J. Agr. Research*, vol. 40, p. 37.
- FORBES, E. B., J. A. FRIES, W. W. BRAMAN AND MAX KRISS 1926 The relative utilization of feed energy for maintenance, body increase, and milk production of cattle. *J. Agric. Research*, vol. 33, pp. 483-492.
- FORBES, E. B., AND MAX KRISS 1932 The analysis of the curve of heat production in relation to the plane of nutrition. *J. Nutrition*, vol. 5, p. 183.
- FORBES, E. B., MAX KRISS AND RUSSELL C. MILLER 1934 The energy metabolism of the albino rat in relation to the plane of nutrition. *J. Nutrition*, vol. 8, p. 535.
- KRISS, MAX, AND RUSSELL C. MILLER 1934 The derivation of factors for computing the gaseous exchange and the heat production in the metabolism of casein by the albino rat. *J. Nutrition* (in press).
- LOWEY, A. 1911 *Die Gase des Körpers und der Gaswechsel*. II. Der respiratorische und der Gesamtumsatz. *Handbuch der Biochemie des Menschen und der Tiere*. Carl Oppenheimer. Bd. 4, S. 277-284.

- LUSK, G. 1928 The elements of the science of nutrition, Philadelphia and London, 4th ed., p. 673.
- MILLER, R. C., AND M. KRISS 1934 The relation of food to the alimentary fill in the albino rat. *J. Nutrition*, vol. 7, p. 627.
- MITCHELL, H. H., AND T. S. HAMILTON 1932 The effect of the amount of feed consumed by cattle on the utilization of its energy content. *J. Agr. Research*, vol. 45, p. 163.
- RUBNER, M. 1902 Die Gesetze des Energieverbrauches bei der Ernährung. Leipzig und Wien, S. 370.
- WIEGNER, GEORG, AND AHMED GHONEIM 1931 Über die Formulierung der Futterwirkung. Ein Beitrag zur Theorie der Verwertung des Unter-nährungs- und Produktionsfutters auf Grund von neuen Fütterungs-versuchen. *Die Tierernährung*, Bd. 2, S. 193-232.
- WILHELMJ, C. M. 1934 A comparative study of the specific dynamic action of the amino-acids alanine and glycine. *J. Nutrition*, vol. 7, pp. 431-445.
- WILHELMJ, C. M., AND J. L. BOLLMAN 1928 The specific dynamic action and nitrogen elimination following intravenous administration of various amino acids. *J. Biol. Chem.*, vol. 77, p. 127.
- WILHELMJ, C. M., J. L. BOLLMAN AND F. C. MANN 1931 A study of certain factors concerned in the specific dynamic action of amino acids administered intravenously and a comparison with oral administration. *Am. J. Physiol.*, vol. 98, p. 1.
- WILHELMJ, C. M., AND F. C. MANN 1930 The influence of nutrition on the response to certain amino acids. *Am. J. Physiol.*, vol. 93, p. 69.
- WILLIAMS, H. B., J. A. RICKE AND G. LUSK 1912 Metabolism of the dog following the ingestion of meat in large quantity. *J. Biol. Chem.*, vol. 12, p. 349.
- WISHART, G. M. 1928 The influence of the protein intake on the basal metabolism. *J. Physiol.*, vol. 65, p. 243.

# THE ENERGY METABOLISM OF THE ALBINO RAT IN RELATION TO THE PLANE OF NUTRITION<sup>1</sup>

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## THREE FIGURES

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## INTRODUCTION

In recent investigations on the energy metabolism of cattle, Forbes, Braman and Kriss ('28, '30) determined, with four steers, the relation of the heat production to the plane of nutrition.

The heat produced by the animal, as related to the food consumed, was found to increase at an accelerating rate from fasting to the maintenance level, and to be higher above than below maintenance; but the rate of increase diminished, in all cases, between the two highest points of observation.

Two attitudes toward this curve of heat production have been expressed by students of nutrition, and two interpretations are implied by procedures which have been employed in the determination of the energy values of rations.

According to one of these positions, the energy cost of utilization of the food, at any level of intake, should be computed by relating the total rise in heat production, above that of fasting, to the quantity of food eaten.

This position, however, seems to the writers to ignore the energy expense of utilization of body nutrients katabolized—first brought to light by Rubner ('02), and the facts as to the

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origin of the heat from different proportions of protein, fat and carbohydrate, as affected by the plane of nutrition, as shown by Forbes and Kriss ('32 a, b).

In accounting for the shape of the curve and for the low heat increments of the food between planes of fasting and maintenance, Forbes, Braman and Kriss ('28, '30), and Forbes and Kriss ('32 a) concluded that the heat production of fasting includes two factors, 1) a net or dynamic factor—a hypothetical minimum value including no waste heat of utilization, and, 2) a waste or thermic factor, representing energy expense of utilization.

This second factor they understand to diminish with rise in the plane of nutrition, from its undermined value at fasting to zero at the point of energy equilibrium.

They recognized that heat increments<sup>2</sup> of food used above maintenance, for body increase, may properly be considered to measure the energy expense of utilization of this part of the total ration; and concluded that heat increments between the lower planes of nutrition are less than the true energy expense of food utilization, by the amount of the waste heat of utilization of body nutrients katabolized.

They further proposed that the most satisfactory method of recognizing the facts as to the significance of the curve of heat production, in the determination of energy values of food, is to compute separate heat increments for maintenance and for body increase—the former based on the heat production of fasting, and the latter on the heat production at the point of energy equilibrium.

Wiegner and Ghoneim ('31) have reported an experiment, with a rabbit, on the utilization of the energy of a mixed diet at five different planes of nutrition, including, among others, fast and one supermaintenance plane. These authors found, by using the fasting katabolism as a measure of the maintenance requirement, and as a base value for the determination of the net energy of the ration at the different levels of

<sup>2</sup>In this paper the term heat increment is used synonymously with the term specific dynamic effect.

intake, a progressive decrease in the percentage utilization of the metabolizable energy of the ration from the lowest to the highest level.

These observations on the rabbit are in substantial agreement with the observations made by Forbes and associates on steers, if the latter are computed in the same way. Wiegner and Ghoneim, however, made no distinction between base value for maintenance and base value for body increase, and they express the opinion that the efficiency of utilization of the ration is determined by the need of the animal for the nutriment.

Møllgaard ('31), in a critical discussion of this subject, took the position that the energy cost of food utilization for body increase is a constant, and that the utilization of the food energy below maintenance is uninfluenced by the amount of food consumed. In defense of his position, he assumed that the results of the experiments of Wiegner and Ghoneim, and of Forbes and associates, which have been cited, especially their fasting katabolism data, are in error, and averred that the reported heat increments based on the fasting data constitute a mathematical illusion. Møllgaard, however, reports no determinations of his own of the heat production of fasting animals.

Mitchell and Hamilton ('32) recently reported observations on the heat production of a single steer at six levels of nutrition, ranging from fast to full feed. The relation between the heat production and the dry matter of food consumed is represented by a zigzag line. This line shows a very slight rise in heat production from fasting to the lowest level of feeding, which was approximately half-maintenance. From this point to higher levels the heat production increased rapidly, but irregularly. Mitchell and Hamilton assign no significance to the irregularities in the increase of heat production above the second level of feeding (approximate maintenance), and characterize the rise from this point to full feed as approximately linear.



If it were true that the heat production above maintenance is a linear function of the amount of food, it would seem illogical to object to the computation of heat increments for body increase with the heat production of maintenance as the base value, but Mitchell and associates do take this position, and have computed the heat increments at the higher levels of feeding by using the heat production of fast as a base value.

Brody and Procter ('33) also compute heat increments, as measures of the energy expense of food utilization, for production as well as for maintenance, with the fasting heat production as the base value—each heat increment for a different level of production, therefore, having a different significance, because each would represent a different proportion of food utilized (at different energy expense for maintenance and for body increase).

In the light of these observations, it appeared desirable to determine the curve of heat production in relation to the quantity of the food, with the albino rat, for comparison with the equivalent curve for cattle, thus to gain further evidence as to the significance of the relationship so expressed.

#### EXPERIMENTAL PROCEDURE

Respiration experiments were conducted with eight male albino rats on a stock diet at three levels of food intake, namely, 8 gm. per day, 6 gm. per day, and 4 gm. per day, and also in the postabsorptive fasting condition. Eight gm. of this diet constituted full-feed, while approximately 5 gm. was required to maintain energy equilibrium. The animals were placed on experiment when they weighed about 100 gm., and full-feed was the first dietary treatment.

The stock diet consisted of a commercial calf-meal which was employed as a conveniently available and approximately complete diet. Its gross composition was: 300 parts linseed oil meal, 400 parts corn meal, 200 parts ground malted barley, 440 parts wheat red dog flour, 240 parts dried skim milk, 300 parts oat flour, 60 parts soluble blood meal, 20 parts salt, 20

parts ground limestone and 20 parts steamed bone meal. The calf-meal was sifted through a 20-mesh sieve, to remove coarse particles. The remaining portion, which alone was used, contained 3.51 per cent N, 2.92 per cent ether extract, 6.24 per cent ash, 10.48 per cent moisture, and 58.42 per cent carbohydrate (by difference). It contained 4047 calories of gross energy and 3145 calories of metabolizable energy per gram. The estimated respiratory quotient of this product was 0.94.

The rats were placed in individual cages of galvanized screen, with false bottoms to prevent coprophagy. The daily amounts of food were given in two equal portions—one at 8 A.M. and the other at 4.30 P.M. The animals were established on the desired plane of food intake prior to the respiration experiments by feeding them at the desired rate for not less than 3 days.

The method of feeding outlined was adopted in this study in the light of the writers' experience in similar studies with cattle, and on account of preliminary experimentation with rats, which showed that when the animals are kept on a restricted diet, and are fed twice during 24 hours, for a number of days, their metabolism becomes fairly constant, and the heat production of a part of the day, under the existing conditions of restricted activity, represents the whole reasonably well.

The respiration experiments were usually of 7 hours' duration. In the experiments in which the animals received feed the rat was placed in the respiration chamber as soon as the morning portion was consumed, which was usually within an hour after the time of feeding. In a few cases when the rats were slow in cleaning up the food, the experimental periods were necessarily shortened.

For the determination of the fasting katabolism, the rat was first established on a plane of approximate energy equilibrium, and the respiration experiment was started 24 hours after the last portion of feed was given.

The sequence of the dietary treatments was as follows: Full feed, one-half feed, three-quarters feed, and fasting. The dates of the respiration experiments, and the initial weights of the animals are presented in table 1.

The apparatus used was devised after the Haldane principle, the moisture and carbon dioxide production, and the oxygen consumption, being determined gravimetrically. Separate measurements on two rats could be made simultaneously. The details of this apparatus are shown in figures 1 and 2.

TABLE 1  
*Schedule of respiration experiments, and body weights*

RAT NO.	8 GM BASAL RATION		4 GM BASAL RATION		6 GM BASAL RATION		FASTING	
	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
	1932	Gm	1932	Gm	1932	Gm	1932	Gm.
457	Nov. 11	111	Nov. 17	101	Nov. 28	116	Dec. 2	107
458	Nov. 11	115	Nov. 16	107	Nov. 28	120	Dec. 2	110
459	Nov. 21	139	Nov. 25	122	Nov. 29	126	Dec. 5	119
460	Nov. 14	123	Nov. 23	111	Nov. 29	121	Dec. 5	110
461	Nov. 15	127	Nov. 23	108	Nov. 30	120	Dec. 6	111
462	Nov. 21	124	Nov. 25	111	Nov. 30	117	Dec. 6	109
463	Nov. 17	117	Nov. 22	103	Dec. 1	114	Dec. 7	106
464	Nov. 16	128	Nov. 22	110	Dec. 1	124	Dec. 8	115

As a means of control of activity, in the interest of comparable results, the rat was placed in a cylinder of  $\frac{1}{4}$  inch mesh galvanized screen, of a fit sufficiently close to prevent the animal from moving about with freedom. The rat in the wire cylinder was then placed in a 1-quart Mason jar; the lid was screwed on loosely, and the jar, rat and wire cylinder were weighed together on a large analytical balance. Weighing to an accuracy of 1 mg. was usually accomplished within 5 minutes. Immediately after weighing, the lid of the jar was replaced by a rubber stopper, provided with connections of rubber tubing for attachment to the ingoing and outcoming air lines of the respiration apparatus. The stoppered jar was placed horizontally in a weighted wooden frame; was im-

mersed in a constant-temperature water bath, maintained at  $28.2^{\circ}\text{C}.$ ; and then the connections were made between the chamber and the air circuit.

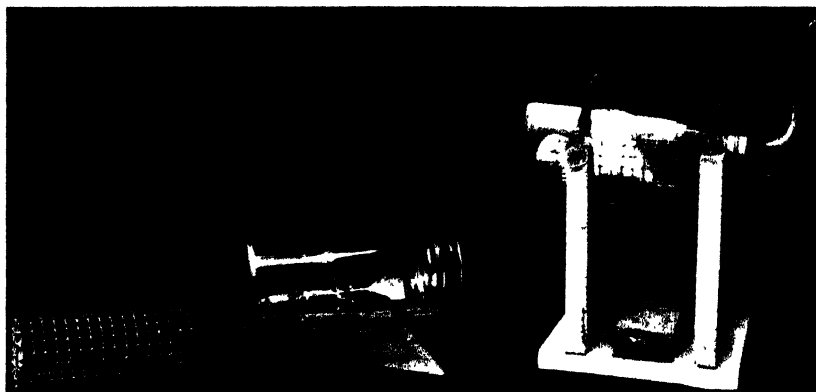


Fig. 1 Mason jar used as a respiration chamber for rats, and a galvanized screen cylinder used to control the activity of the animal.

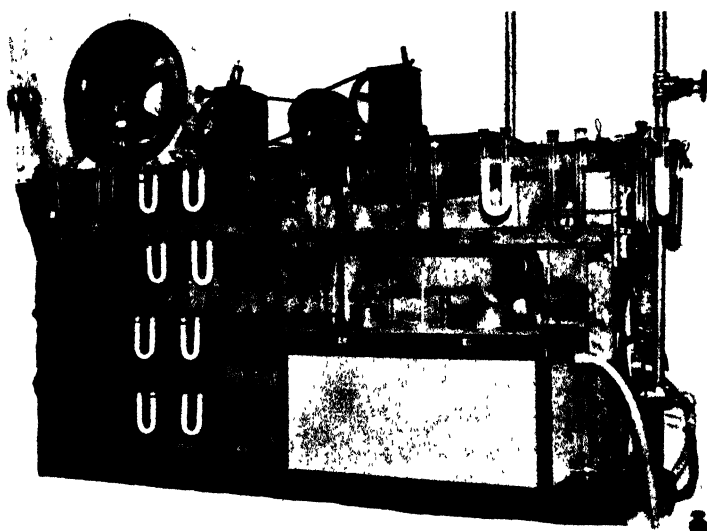


Fig. 2 Respiration chamber and accessory equipment, for indirect calorimetric experimentation with rats.

The water bath was covered by a lid in the center of which was a 25-watt electric light so placed as to illuminate the chamber below it. The purpose of the light was to incline the rat to keep its eyes shut, and therefore to remain quiet.

No observations were made of the temperature of the air in the chamber, but it is safe to assume that it was only slightly higher than that of the surrounding water, and that it was above the critical point during all metabolism measurements, 28°C. having been found by Benedict and MacLeod ('29) to be the critical temperature for the fasting rat. Outside of the respiration apparatus the rats were kept in a room at a temperature of about 27°C., which was exceeded only in very warm weather.

The rate of ventilation was maintained, during this series of experiments, at 35 to 40 liters per hour, by means of a double-stage rotary pump, designed primarily for vacuum work; and the air supply was measured by a Bohr meter. The rate of ventilation was maintained constant during experiments, in order to minimize possible differences in composition of the air in the chamber at the end as compared with the beginning of experimental periods.

The carbon dioxide was collected in U-tubes containing soda lime, and moisture was collected in U-tubes containing sulphuric acid and finely divided pumice stone. Two sets of such absorption tubes were connected with the outcoming air line from the chamber, to be used alternately, 1 hour at a time, except that at noon one set of tubes was used for 2 hours, as a matter of convenience. The length of experiments, with some exceptions, was 7 hours.

The oxygen consumption during experimental periods was determined as the difference between the loss in weight of the animal in the chamber and the gain in weight of the absorption tubes, due to the moisture and carbon dioxide produced by the animal. Respiratory quotients were determined from the total carbon dioxide produced and the total oxygen consumed, the error in using total instead of non-protein respiratory quotients having been found practically negligible.





The heat production was computed from the average respiratory quotient for the experimental period and the hourly carbon dioxide production. The weight of carbon dioxide of the first hour, and sometimes that of the second hour also, was omitted from the computation of the heat (but not from the computation of the R. Q.'s) because of the accumulation of carbon dioxide which occurred in the chamber while the animal was being weighed. The errors in the heat production due to the use of the average respiratory quotients are considered to be relatively small, in view of the fact that under the experimental conditions no very large variations in the respiratory quotients could be expected, especially, in consideration of the intervals between feeding and of the observed rates of  $\text{CO}_2$  production.

#### DISCUSSION OF RESULTS

Table 2 gives the hourly heat production in consecutive order and the total respiratory quotients for the individual rats in the different experimental periods. For the purposes of comparison and statistical treatment, all of the data for heat production are expressed as calories per 100 gm. of empty weight, this basis of reference being considered more significant than the gross body weight.

The determination of the empty weight of the experimental animals, by the method of Miller and Kriss ('34), was based on parallel determinations on control animals which received the same dietary treatment. Thus it was assumed that the initial empty weights of the experimental subjects with food intake of 8 gm., 5.5 gm., and 4 gm., and with no food, were 88.74 per cent, 89.98 per cent, 90.58 per cent, and 96.93 per cent, respectively, of the gross weights. The proportion of empty to gross weight of rats which received 6 gm. was assumed to be the same as with rats which received 5.5 gm. of food.

Since the empty weight of each of the animals was about 100 gm. (table 1), the computation of the heat production to the basis of 100 gm. empty weight involved but small cor-



TABLE 2

*Hourly heat production of male rats, per 100 gm. of empty body weight, and total respiratory quotients, as influenced by the plane of nutrition*

RAT NO.	FASTING		4 GM. BASAL RATION PER DAY		6 GM. BASAL RATION PER DAY		8 GM. BASAL RATION PER DAY	
	<i>Oals.</i>	<i>R.Q.</i>	<i>Oals.</i>	<i>R.Q.</i>	<i>Oals.</i>	<i>R.Q.</i>	<i>Oals.</i>	<i>R.Q.</i>
457	596	0.75	727	0.89	825	0.96	848	0.90
	601		632		777		851	
	547		632		653		701	
	547		681		653			
	575		604		697			
	653				711			
458	544	0.73	688	0.88	653	0.98	912	0.93
	497		584		666		912	
	546		643		701		788	
	547		643		701		738	
	653		691		668		858	
	539		625		673			
459	538	0.75	701	0.90	787	0.99	892	1.00
	569		657		699		885	
	587		603		652		885	
	587		603		652		805	
	535		668		647		749	
			668		777			
460	464	0.74	618	0.94	688	0.99	804	1.01
	488		625		702		774	
	486		547		703			
	486		547		643			
	534		577		736			
			609					
461	605	0.74	632	0.92	705	0.98	905	0.93
	621		718		645		936	
	490		606		611		810	
	490		606		611			
			619		649			
			636		652			
462	524	0.72	564	0.89	704	0.98	757	1.01
	500		538		600		790	
	477		538		606		721	
	477		625		606		721	
	525		583		679		718	
							785	
463	478	0.74	548	0.93	661	1.01	755	0.96
	534		548		626		772	
	461		535		626		823	
	461		558		656			
	447							
	450							
464	550	0.75	615	0.91	626	0.95	980	0.91
	475		589		644		980	
	511		589		645		996	
	511		586		604		785	
	575		594				781	
	520							
Mean	530 ± 5.4	0.74	614 ± 5.1	0.91	672 ± 5.31	0.98	826 ± 9.6	0.96
Standard deviation of means	8.0 ± 0.59		7.6 ± 0.56		7.9 ± 0.59		14.3 ± 1.22	
Standard deviation	52.5 ± 3.87		49.8 ± 3.67		51.2 ± 3.83		80.9 ± 6.90	

rections, and was considered satisfactory for the purposes in view.

The surface area law, and the exponential relationship between body weight and metabolism, were recognized by computing the corrections for the differences between the actual empty weight and the standard weight of 100 gm., not in direct proportion, but in proportion of the two-thirds power of these weights.

The data of table 2 reflect the effectiveness of the experimental technic. The heat measurements for consecutive hours were fairly uniform, and a satisfactory degree of uniformity was also found among the respiratory quotients. Of the factors which have contributed to the uniformity of these measurements, the following deserve especial emphasis:

1. Restriction of the activity of the rat while in the respiration chamber. All who have attempted to use rats for respiration experiments recognize that the activity of the subjects constitutes a serious problem. Some workers have attempted to overcome this difficulty by making records of the activity of the animals, to serve as a basis of selection among the experimental data, but for obvious reasons this is not a satisfactory solution. On the other hand, the limitation of space for the rat in the chamber, and the bright illumination, in the experiments here discussed, were found highly satisfactory as a means of placing the metabolism measurements on a comparable basis. It is true that when first inclosed in the cylinder, a rat is restless; but it soon accommodates itself, and then remains remarkably quiet.

2. Establishing the animals on the desired plane of food intake prior to the respiration experiments. The determination of dynamic effects, or heat increments, from established planes of feeding is equivalent to the determination of the distance between two parallel lines (representing the heat production on two planes of nutrition), which may be accomplished readily and with certainty. In other words, the heat production is not curvilinear, but is practically on a level. This procedure, which has been employed in all determina-

tions of heat increments with cattle at this institute, has marked practical advantages over the determination of the heat increment due to a single feeding.

3. Feeding twice a day. The advantage of dividing the daily ration into two equal portions, and feeding one in the morning and one in the evening, is twofold: The food is eaten promptly, thus making it possible to conduct the heat measurement at a convenient, predetermined time; and, what

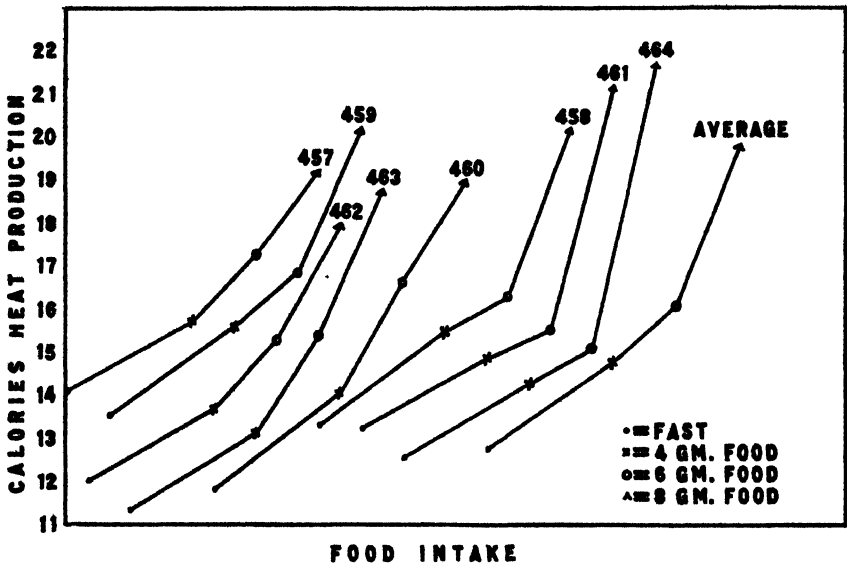


Fig. 3 The heat production of albino rats related to the food intake.

is more important, this method of feeding makes for uniformity of metabolism throughout the day.

In figure 3 are plotted the calories of heat production of the individual rats, computed to a 24-hour basis (table 3) against the grams of food intake per day. The average results from the eight animals, similarly plotted, are represented in this figure by a separate curve which is considered as the most significant. Each of these curves shows a greater rate of increase in metabolism from the 6-gm. to the 8-gm. level than from the fasting to the 4-gm., or the 6-gm., or the 8-gm. level.

With one exception (rat no. 460), the increase from the 6-gm. to the 8-gm. level was greater than from the 4-gm. to the 6-gm. level. Five gm. of food supplied the approximate energy maintenance requirement of the animals.

TABLE 3

*Daily heat production of individual rats per 100 gm. of empty body weight, on different planes of nutrition*

RAT NO.	FASTING	4 GM. BASAL RATION	6 GM. BASAL RATION	8 GM. BASAL RATION
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
457	14,088	15,720	17,256	19,200
458	13,296	15,504	16,248	20,208
459	13,512	15,600	16,848	20,232
460	11,808	14,088	16,656	18,936
461	13,248	14,880	15,504	21,216
462	12,024	13,680	15,336	17,976
463	11,328	13,128	15,408	18,792
464	12,576	14,280	15,120	21,696

The average curve representing the relationship between the plane of nutrition and the heat production shows a striking similarity to the corresponding curves obtained by Forbes and associates with steers. Of particular interest is the rather marked break in the curve at about the maintenance level. The average numerical values for the heat increments, per gram of food, between various levels (table 4), bring out this point even more strikingly.

TABLE 4

*Average daily heat production of rats on different planes of nutrition, and the resulting heat increments*

FOOD INTAKE	HEAT PRODUCTION PER DAY PER 100 GM. OF EMPTY BODY WEIGHT	PLANES OF NUTRITION COMPARED	HEAT INCREMENTS PER GRAM OF FOOD
	<i>Cals.</i>		<i>Cals.</i>
Fasting	12,720	Fast and 4 gm. basal ration	504
4 gm. basal ration	14,736	Fast and 6 gm. basal ration	568
6 gm. basal ration	16,128	Fast and 8 gm. basal ration	888
8 gm. basal ration	19,824	4 gm. and 6 gm. basal ration	696
		4 gm. and 8 gm. basal ration	1272
		6 gm. and 8 gm. basal ration	1848

If the fasting heat production is used as the base value for the computation of the heat increments, these values rise progressively from 504 calories per gram of food eaten, at the 4-gm. level, to 888 calories at the 8-gm. level; but when the heat increments are computed between consecutive points of observation, the increase from the 4-gm. to the 6-gm. level is 696 calories; and from the 6-gm. to the 8-gm. level is 1848 calories per gram.

In comparing these curves for rats with the equivalent curves for steers, it should be borne in mind that at the highest planes of nutrition the steers ate from two to three times as much feed as required for maintenance, whereas the rats ate a maximum of only about one and three-fifths times as much feed as required for maintenance. It seems, therefore, that they did not eat enough, and that there were not points of observation sufficient in number, to show the slight reverse curvature that was found at the higher planes of nutrition with each of the four steers used as subjects, this reverse curvature having been interpreted as the result of diminished metabolizability of the food at the higher planes of nutrition.

It would be unilluminating and, indeed, unwarranted to assume, as did Møllgaard ('31) that errors in the determination of the fasting katabolism of steers, by Forbes and associates, are the chief cause of the relatively low heat increments, as these are computed with reference to the fasting heat production. Møllgaard's assertion that these determinations of the fasting heat production were too high, on account of food residues in the alimentary tract, was not based upon significant evidence, and finds no support in the analysis of the heat production of these animals by Forbes and Kriss ('32 a, b) which showed that in the fasting periods the non-protein material katabolized consisted entirely of fat.

Opposed to Møllgaard's assertions that the determinations of fasting heat production of steers, by Forbes and associates, are too high, and that values computed with reference to these determinations constitute only a mathematical illusion, are other experimental data of Forbes, Braman, Kriss

and Swift ('31), and analogous data of Benedict and Ritzman ('27), representing the fasting katabolism of steers, and the corresponding respiratory quotients, which show that the true fasting status is reached, with cattle, between the second and the fourth day of fast.

The values alluded to by Møllgaard as illusory are illusory only if misunderstood and misinterpreted. The experimental data of Forbes and associates, with steers as subjects, are characterized, in the writers' opinion, by a high degree of validity, and it is their belief that when the contribution to the curve of heat production of the dynamic effects of body nutrients katabolized is correctly appreciated it will be understood that heat increments for maintenance, as computed with reference to the fasting heat production do not express the true, physiological, energy expense of food utilization, but are in reality conventional values, and are so computed only because there is no other base value representing the status of no-feed-intake which is as definite as is a properly standardized determination of the heat production of fast. Forbes, Braman, Kriss and Swift ('31) have adopted terms for such a standardization.

There is an apparent inconsistency in Møllgaard's criticism ('31) of Wiegner and Ghoneim's data dealing with the effect of the plane of nutrition on the utilization of the metabolizable energy of the ration, since, on the one hand, he recognized the finding of a greater energy cost of food utilization (or a lower net energy value) for body increase than for maintenance; but, on the other hand, assuming in this case also, as in the work of Forbes and associates with steers, that the value for the fasting katabolism of the experimental animal (rabbit) was too high, he recalculated the data in such a way as to show that there is no difference in the efficiency of utilization of the energy of the ration at levels above and below the maintenance level.

That the metabolism data for the fasting rats (table 2) represent true fasting conditions is shown by the facts that the total respiratory quotients are between 0.72 and 0.75 (the

non-protein respiratory quotient would be slightly lower), and that practically no food residue was found in the stomachs and small intestines of control animals, in a status of fast corresponding to that of the subjects of the respiration experiments. The relatively low heat increments obtained in comparisons involving the fasting heat production (table 4), therefore, must be accepted as matters of fact, but they must not be interpreted as representing the true energy expense of food utilization.

The results of this study confirm, in a general way, the relations between the quantity of feed eaten, and the heat produced, as determined by Forbes and associates with cattle, and by Wiegner and Ghoneim with the rabbit. There is also some evidence, in work of Gigon ('11), that the relationship of food to heat production is quite similar in the case of man. It seems likely, therefore, that the same physiological principles prevail, in this relation, in these different species.

Experimental work bearing on the specific causes for the apparent differences in the energy cost of food utilization at different planes of nutrition will be discussed in subsequent papers.

#### SUMMARY AND CONCLUSIONS

Respiration experiments, by the open-train Haldane procedure, were conducted with eight male albino rats, of approximately 100 gm. empty body weight, at four planes of nutrition, ranging from fasting to full feed. The same diet—a commercial, mixed calfmeal—was used throughout. The rats were fed twice daily, and were established on the desired plane of food intake prior to the respiration measurements. The measurement of the fasting metabolism was started 24 hours after the last meal was given.

The activity of the rat was restrained, during respiration experiments, by inclosure in a rather close fitting galvanized screen cylinder within the chamber.

The hourly heat production and the total respiratory quotients varied but little for any given dietary treatment.

The average daily heat production per 100 gm. of empty body weight was 12,720 calories for fasting, 14,736 calories with 4 gm. of food; 16,128 calories with 6 gm. of food, and 19,824 calories with 8 gm. of food per day.

The average increments in heat production between fast and the 4-, 6- and 8-gm. food levels, respectively, were 504, 568 and 888 calories per gram of food consumed. Much higher heat increment values were obtained in comparisons which did not involve the fasting heat production. The highest value, 1848 calories per gram, was obtained between the two highest levels of feeding, which were above maintenance.

The results of this study confirm the observations of Forbes and associates on steers, of Wiegner and Ghoneim on the rabbit, and of Gigon on man, in showing a progressive increase in the rate of rise of the heat production with increase in food consumption, within certain limits. These results and others refute Møllgaard's allegation that the comparatively low heat increments between the fasting and the maintenance planes, found by Forbes and associates, and by Wiegner and Ghoneim, are due to experimental errors in the fasting heat measurements.

The same general relationship apparently exists between food consumption and heat production in the rat, the rabbit, the steer, and the human being.

#### LITERATURE CITED

- BENEDICT, F. G., AND GRACE MACLEOD 1929 The heat production of the albino rat. II. The influence of environmental temperature, age, and sex: comparison with the basal metabolism of man. *J. Nutrition*, vol. 1, pp. 367-398.
- BENEDICT, F. G., AND E. G. RITZMAN 1927 The metabolism of the fasting steer. Carnegie Institution of Washington, pub. 377, pp. 1-246.
- BRODY, S., AND ROBERT C. PROCTER 1933 The influence of the plane of nutrition on the utilizability of feeding stuffs. *Mo. Agr. Expt. Sta., Research Bull.* 193, pp. 5-48.
- FORBES, E. B., W. W. BRAMAN AND MAX KRISS 1928 The energy metabolism of cattle in relation to the plane of nutrition. *J. Agr. Research*, vol. 37, pp. 253-300.
- 1930 Further studies of the energy metabolism of cattle in relation to the plane of nutrition. *J. Agr. Research*, vol. 40, pp. 37-78.



- FORBES, E. B., W. W. BRAMAN, MAX KRISS AND R. W. SWIFT 1931 The fasting metabolism of cattle as a base value of heat production in the determination of the net energy of feeding stuffs. *J. Agr. Research*, vol. 43, pp. 1003-1014.
- FORBES, E. B., AND MAX KRISS 1932 a The analysis of the curve of heat production in relation to the plane of nutrition. *J. Nutrition*, vol. 5, pp. 183-197.
- FORBES, E. B., AND MAX KRISS 1932 b Der Energiewechsel des Rindes in Beziehung zur Grösse der Nahrungsaufnahme. *Die Tierernährung*, Bd. 4, S. 215-239.
- GIGON, ALFRED 1911 Über den Einfluss der Nahrungsaufnahme auf den Gaswechsel und Energieumsatz. *Arch. f. d. ges. Physiol. des Menschen u. d. Thiere*, Bd. 140, S. 509-592.
- MILLER, R. C., AND MAX KRISS 1934 The relation of food to alimentary fill in the albino rat. *J. Nutrition*, vol. 7, pp. 627-632.
- MITCHELL, H. H., AND T. S. HAMILTON 1932 The effect of the amount of feed consumed by cattle on the utilization of its energy content. *J. Agr. Research*, vol. 45, pp. 163-191.
- MØLLGAARD, H. 1931 Zur Frage der Abhängigkeit des Nährwertes von der Futtermenge. *Die Tierernährung*, Bd. 2, S. 520-529.
- RUBNER, M. 1902 *Die Gesetze des Energieverbrauchs bei der Ernährung*, S. 370. Leipzig und Wien.
- WIEGNER, GEORG, AND AHMED GHONEIM 1931 Über die Formulierung der Futterwirkung. Ein Beitrag zur Theorie der Verwertung des Unter-nährungs- und Produktionsfutters auf Grund von neuen Fütterungsversuchen. *Die Tierernährung*, Bd. 2, S. 193-232.

# THE EFFICACY OF VITAMIN D ADMINISTRATION IN AQUEOUS PREPARATION

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## FOUR FIGURES

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## INTRODUCTION

For experiments which we were conducting in 1931 on in-vitro calcification of bone we found it necessary to make aqueous preparations of vitamin D. The following series of experiments was arranged to determine the efficacy of these preparations on growing animals rendered appropriate for the purpose by a diet deficient in calcium as well as in phosphorus. We have used the rat for all our studies whether of the living animal or of bone fragments in-vitro despite the general conviction that the rat, during its growing period, is relatively less dependent than the growing child upon vitamin D, for these studies are designed to inquire into the principle rather than the degree of dependence.

The activated ergosterol used in the experiments was generously provided by Messrs. Mead, Johnson & Co. The actual feeding and medication were carried out under our immediate supervision by Miss Dora Steinfeld who also made some of the ash determinations quoted in this essay.

The mineral-deficient diet used was the following:

	<i>parts</i>
Dried yellow corn	76
Wheat gluten	20
Talcum purificatum	3
Sodium chloride	1

Our determinations show a calcium content of 0.74 per cent in the ash of this diet compared with 35.3 per cent in the ash of the Steenbock and Black diet no. 2965. Therefore the lime in our special diet seems negligible. The diet will be defined in the following pages as the mineral-deficiency diet to distinguish it from the regular rachitic diets no. 2965 of Steenbock and Black and no. 3143 of McCollum, both of which were

TABLE 1

*Rats weaned at 30 days were fed on the mineral-deficiency diet for the preparatory period and then utilized in the following experiments*

LITTER	NUMBER OF RATS	PREPARATION PERIOD	TYPE AND PERIOD OF MEDICATION	
		<i>days</i>		<i>days</i>
I	9	8	Aquasterol F <sup>a</sup>	21
II	7	16	Aquasterol F <sup>a</sup>	21
III	7	8	Talc residue <sup>b</sup>	21
IV	7	8	Viosterol <sup>c</sup>	21
VI, VII	17	21	Aquasterol G <sup>d</sup>	32
IX, X, XI	19	35 to 39	Aqueous ergosterol <sup>e</sup>	33
XII, XIII, XIV	14	27	Aquasterol H <sup>f</sup>	25

<sup>a</sup> Ten cc. 10,000 X viosterol extracted with water to 75 cc.; dosage, 1 cc. daily.

<sup>b</sup> Ten cc. 10,000 X viosterol extracted with water to 250 cc.; dosage, 1 cc. daily.

<sup>c</sup> Talc residue left after extraction of F<sup>a</sup> suspended in 75 cc. water; dosage, 1 cc. daily.

<sup>d</sup> One gm. 10,000 X viosterol diluted to 25 gm. with maize oil; dosage, 5 minims (1000 r.u.) daily.

<sup>e</sup> One-tenth mg. crystalline activated ergosterol incorporated in 250 cc. water; dosage, 1 cc. daily.

<sup>f</sup> An aqueous preparation of commercial cod liver oil U.S.P.; dosage, 1 cc. daily (1 cc. aquasterol H contains the water soluble extractives of 1 cc. C.L.O.).

designed to disturb the relation between calcium and phosphorus, whereas our intention was to eliminate both minerals. On the basis of experiments by McCollum, Kruse and Orent ('33), it may be surmised that the magnesium content of the diet, in short term experiments, will prevent the elimination of mineral from the skeleton. The absence of calcium and phosphorus from the diet will greatly restrict the amount of these minerals available for the growth of the animals.

The experiments were all conducted in the following manner. White rats on weaning at 1 month of age were fed on the mineral-deficiency diet for an initial preparatory period of varying duration (table 1). They were placed singly or in pairs in metal containers covered with wire netting and black paper. The diet, finely ground, and in sufficient quantity, with fresh drinking water, was at all times available to the animals. The experimental medication was administered through a special pipette to insure adequate consumption and to control the dosage. Control litter mates were kept on the mineral-

TABLE 2  
*Bone ash determinations: stock diet*

LITTER	DAYS AFTER BIRTH	DAYS AFTER WEANING	PER CENT ASH
XVI (8 rats)	13		25.8
	19		29.7
	27		34.3
	Weaned at 30 days		
	35	5	33.8
	42	12	38.1
	53	23	38.2
	53	23	38.6
	72	42	41.0
XVII (4 rats)	16		26.4
	23		31.9
	Weaned at 30 days		
	44	14	35.2
	75	45	43.0

deficiency diet without medication. The upper end of one tibia was split longitudinally and one-half used for histological study (Shipley et al., '20, '21). The remainder of both lower extremities (femora, tibiae and fibulae but not the feet) were dissected out, cleaned, dehydrated and defatted, then ashed to constant weight. The results are expressed as percentage of ash in dried, fat-extracted bone (Hume et al., '32).

Animals taken from litters VIII and XIV, two litters fed continuously on the deficiency diet without medication, give the ash determinations of table 3 which stand in contrast to

the figures in table 2, the ash determinations on litters XVI and XVII fed on a contrasting stock diet, made up as follows:

	<i>parts</i>
Whole wheat flour	66
Whole milk powder	22
Talcum purificatum	1
Sodium chloride	1
Lettuce, three times weekly	

These figures show that the several litters differ greatly in their skeletal ash percentage but nevertheless the different members of a single litter, despite segregation and allowing

TABLE 3  
*Bone ash determinations: deficiency diet*

LITTER	DAYS AFTER WEANING	PER CENT ASH
XIV (7 rats)	18	34.6
	19	34.2
	30	31.6
	31	29.1
	31	30.2
	49	25.6
	55	26.1
VIII (3 rats)	30	25.8
	40	24.1
	45	24.8

for the individual differences in efficiency of food utilization (Palmer and Kennedy, '31), indicate the progress of skeletal mineral content.

While preparing this report for publication, we have noted the corresponding but apparently less detailed study by Becari ('33) on the efficacy of water-soluble derivatives of vitamin D in the prevention and cure of rickets experimentally produced in white rats by McCollum diet 3143.

We have also received a copy of the report by Bourdillon and his associates ('31). This excellent study points out many pitfalls but it does not specially emphasize the fact that the rats under observation were kept upon the rachitogenic diet 2 weeks only before commencing the vitamin medication.

Our histological observations lead us to lay special stress upon this duration for we find that the bones of rats fed upon a rachitogenic diet for 19 days or more are already so pathological that the healing process can do no more than repair an area so disorganized that the structure remains permanently mutilated. Another feature to which we would draw attention is the progressive rarefaction of the shaft which can be seen on the roentgenographic scale of healing. The production of this rarefaction is discussed a little later in our report.

#### DETAILS OF EXPERIMENT

In the experiments recorded below are two series. The first inquires into the action of that fraction of activated ergosterol-in-oil which can be transferred to watery solution. For purposes of distinction this type of preparation is recorded as aquasterol and the letter of designation defines the concentration. The second series inquires into the action of activated ergosterol in crystalline form when utilized in watery solution.

Aquasterol F was obtained by 'extracting' 10 cc. of 10,000 X viosterol with water to a final volume of 75 cc. One cc. of this preparation was administered daily to each animal of two litters of rats (litters I and II, table 4). Litter I was fed on the mineral-deficiency diet for 8 days; litter II for 16 days. Thereafter, to both litters, medication was given along with the mineral-deficiency diet for 21 days. The animals serving as negative controls and receiving no medication show rickets by both histological and x-ray examination. The animals of litter I show no rickets after their 8-day preparation but those of litter II show early rickets at the end of 16 days before commencement of the aquasterol experiment. After 21 days of medication there is no histological evidence of rickets in any of the test animals and the degree of healing can unhesitatingly be assessed at 6 on Dyer's histological standards ('31).

The residual viosterol left from the preparation of aquasterol F was suspended in water and made up to a volume of

75 cc. One cc. of this suspension was administered daily by mouth to each animal of another litter (litter III, table 5).

TABLE 4

*Bone ash determinations: deficiency diet from date of weaning; medication by aquasterol F*

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
I (9 rats)	7	0	22.7
	9	1	22.2
	16	8	28.9
			31.4
			30.3
			28.4
	29	21	29.7
			36.6
			38.4 Ave. 32.5
II (7 rats)	14	0	26.4
			26.7
	27	11	35.3
			40.1
			39.6
	37	21	40.7
			40.3

TABLE 5

*Bone ash determinations: deficiency diet from date of weaning; medication by talc residue*

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
III (7 rats)	10	2	20.2
	12	4	20.2
	21	13	31.2
	21	13	35.0
	23	15	32.0
	27	19	31.7
	29	21	31.7

The preparatory period during which the animals received the deficiency diet and the period of medication were identical with those of litter I. At no time during the 21 days did any of these test animals show evidence of rickets.

For comparison with the foregoing experiment litter IV (table 6) was fed upon the mineral-deficiency diet for 8 days. Then for 21 days these animals received daily, in addition to the mineral-deficiency diet, 5 minims of a preparation (representing 1000 rat units of vitamin D) obtained by diluting 1 gm. 10,000 X viosterol to 25 gm. with maize oil.

Comparing tables 4, 5, 6 we find that ash content progressively increases in approximately identical fashion no matter whether the vitamin D is administered as an aqueous preparation (aquasterol F) or in oily solution (talc residue, viosterol dilution).

TABLE 6

*Bone ash determinations: deficiency diet from date of weaning; medication by viosterol*

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
IV (7 rats)	16	8	29.9
			26.9
			32.3
			33.5
	29	21	29.8
			30.0
			29.9 Ave. 30.4

The histological features of the tibiae studied on test animals from these three litters are practically identical. That is to say, restoration of the typical structure of healthy growing bone is facilitated by administration of vitamin D in any form. We shall have more to say about the mineralization of the bone itself.

A weaker preparation, aquasterol G, was obtained by 'extracting' 10 cc. of 10,000 X viosterol with water to a final volume of 250 cc. The two litters of rats (VI and VII) used in this experiment were maintained on the mineral-deficiency diet for 21 days from the date of weaning. From the twenty-second to the fifty-third day, inclusive (32 days in all), each rat received daily 1 cc. of aquasterol G by dropper. In this experiment the increase in skeletal ash-content (table 7) though small and variable, is nevertheless distinct. The



histological preparations demonstrate progressive restoration so that, at the end of the experiment, the bones of each surviving animal showed an approximately normal growth zone.

Figures 1 to 4 represent the histological changes in litters VI and VII which received the more dilute preparation (aquasterol G) after a preparatory period of 21 days on the mineral-deficiency diet. These figures, while illustrative of

TABLE 7

*Bone ash determinations: deficiency diet from date of weaning; medication by aquasterol G*

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
VI (9 rats)	21	0	29.5
	24	3	26.7
	29	8	28.7
	37	16	26.8
	37	16	25.6
	45	24	31.1
	45	24	29.1
	53	32	28.0
	53	32	32.8
VII (8 rats)	21	0	28.1
	33	12	30.6
	33	12	27.0
	37	16	28.6
	45	24	29.4
	45	24	31.4
	53	32	28.0
	53	32	34.5

the several other experiments reported in this communication, are particularly significant because they indicate what is really happening to a bone lacking in adequate mineral supply but under the influence of vitamin D.

In figure 1 the mineral-deficiency diet during 21 days has resulted in a generally defective mineralization quite characteristic in its features. The compacta is thin; the spongiosa is but partly mineralized so that there are areas of osteoid tissue interspersed among the bony trabeculae. This results

in a lattice-like architecture quite readily demonstrable by roentgenography. The distal margin of the spongiosa is irregular so that osteoid masses break the uniformity of its line. Beyond this is the zone of hypertrophic cartilage totally devoid of mineralization and even showing cystic areas. Beyond this again is the zone of proliferative cartilage also devoid of mineralization and indistinguishable from the diaphyso-epiphysial plane which appears unaffected. The bony epiphysis shows a demineralization comparable with that



Fig.1 Upper extremity tibia, negative control. Mineral-deficiency diet 21 days. No medication. Poor mineralization of compacta and spongiosa in both shaft and epiphysis. Areas of osteoid tissue interspersed between trabeculae of spongiosa. Cyst formation. Absence of mineralization in zones of hypertrophic and proliferative cartilage. Diaphyso-epiphysial plane apparently unaffected.

of the shaft but no other abnormal condition. It is true that this description differs from that usually presented in the literature. The difference is not in fact but in terms. We believe that an adequate anatomical analysis is essential to a proper interpretation of the phenomena accompanying experimental studies of dietetic deficiencies.

Figure 2 shows the change evident after 8 days of medication (1 cc. aquasterol G daily) during which the mineral-deficiency diet was continued. The compacta is thinner and the spongiosa more defective in mineralization. The area of

hypertrophic cartilage shows a mineralization of characteristic pattern (Dodds, '32) differing completely from the trabecular ossification of the spongiosa and capping the margin of the spongiosa by a uniform unbroken line of mineralization. The area of proliferative cartilage is not completely penetrated by mineral and therefore, although the clear area is narrowed it is not reduced to the thickness of the diaphyso-epiphysial plane which, with the adjacent tissue of



Fig. 2 Upper extremity tibia. Mineral-deficiency diet 29 days, supplemented during the last 8 days by 1 cc. of aqueous preparation of vitamin D (aquasterol G) daily. Compacta and spongiosa more poorly mineralized. Irregularity of spongiosa covered by unbroken cap of mineralization in zone of hypertrophic cartilage. Diaphyso-epiphysial plane remains unaffected.

the bony epiphysis, remains apparently unaffected. Of course demineralization is evident in the spongiosa of the epiphysis as in the spongiosa of the shaft.

Figure 3 illustrates the approximately normal healthy appearance of the growth area after 12 days of medication. Restoration has taken place at the expense of spongiosa in shaft and epiphysis which are more demineralized than before. The compacta is now quite defective in places; the spongiosa shows large areas devoid of mineralization and recognized as osteoid tissue. The zone of proliferative carti-

lage is almost wholly transformed into a zone of provisional calcification. There are no cystic areas and the march of mineralization toward the margin of the diaphyso-epiphyseal plane is clearly seen.

Figure 4 demonstrates the maintenance of a fairly healthy growth zone even after 53 days of the deficiency diet, on the last 32 of which the medication was given daily. Compacta and spongiosa are more defective than ever in mineralization.



Fig. 3 Upper extremity tibia. Deficiency diet 33 days, supplemented during the last 12 by 1 cc. aquasterol G daily. Compacta and spongiosa still more poorly mineralized. Zone of proliferative cartilage also partly mineralized so that clear area adjacent to diaphyso-epiphysial plane is reduced in thickness. Approximate restoration of normal growth area at expense of mineralization in epiphysis and shaft.

The partial mineralization of the zone of hypertrophic cartilage is now maintained with difficulty. The clear area comprising diaphyso-epiphyseal plane and adjacent zone of proliferative cartilage is now wider again owing to relative failure of penetration of the latter by the mineral salts. The uniformity of mineralization of the hypertrophic zone adjacent to the spongiosa is still maintained unbroken but it now forms merely a thin cap on the erratically ossified spongiosa.

This figure is particularly significant for it indicates the progressive constitutional strain incident to an attempt, by means of vitamin medication, to maintain a normal bony architecture despite the continuance of mineral deficiency.

Three litters (XII, XIII, XIV) consisting of fourteen animals were utilized in a test of aquasterol H made by 'extracting' with water an ordinary grade of commercial cod liver oil so that each cubic centimeter of the product contains the



Fig. 4 Upper extremity tibia. Deficiency diet 53 days, supplemented during the last 32 by 1 cc. aquasterol G daily. Very defective mineralization of compacta and spongiosa. Mineralization of proliferative cartilage zone lost and that of hypertrophic zone failing so that clear area adjacent to diaphyso-epiphysial plane is again widening.

water-soluble extractives of 1 cc. of cod liver oil. One cc. of this preparation was fed daily for 24 days to each of the four test animals in litter XIII after it had been subjected to a preparatory period of mineral-deficiency feeding lasting 27 days from the date of weaning. Litter XII was maintained on the deficiency diet for a period of 27 days after weaning but, thereafter, as a control to litter XIII, the deficiency diet was supplemented by 1 cc. daily of the stock cod liver oil from which aquasterol H was obtained. Litter XIV was main-

tained on the deficiency diet throughout the experimental period.

Histological studies of litter XII showed commencing restoration of the growth area after 4 days of medication (Bills et al., '31, p. 633) and approximately normal structure after 13 days. Litter XIII showed, at 13 days, a histological picture similar to that shown by litter XII at 4 days. It is evident then that the efficacy of aquasterol H was roughly one-third that of the cod liver oil. Ash determinations from these litters are recorded in tables 3, 9. Once again the histological picture is restored to an approximate normal by the water-soluble fraction of the antirachitic principle (vitamin D) even though the mineral of the skeletal depot is relatively deficient as shown by the bone-ash values.

For the second series of experiments an aqueous solution of crystalline activated ergosterol was obtained by dissolving 0.1 mg. of the crystalline substance in 250 cc. of water. One cc. of this preparation was fed daily to each test animal of litters IX, X, XI which were maintained upon the deficiency diet for 35 to 39 days after weaning and before administering the medication.

To judge from comparison with the figures from control animals segregated at the foot of the columns in table 8 mineralization of the skeleton is retained much better in the presence of vitamin D than without it. The histological pictures are comparable with those already described, and from them it is evident that medication administered for 6 days in this experiment restores in part the healthy appearance of the growth area while medication for 9 days approximately completes the restitution. Nevertheless vitamin D, though it directs the disposition of mineralization, cannot secure an actual increase of mineralization in the absence of an adequate supply. That the ash content of the bones in the protected animals was maintained indicates that just as vitamin D directs available calcium to the growth area so it must have some function in directing surplus calcium, wherever found, into the skeletal depots. This is probably what is meant by the assumed power of vitamin D to strengthen bones by in-

creasing their mineralization. Where the mobilization of this calcium takes place is not a problem for our present attention.

Maintenance of ash percentage also means that velocity of growth in the bone is not disproportionate to the available mineral during the 33 days of medication.

TABLE 8

*Bone ash determinations: deficiency diet from date of weaning; medication by ergosterol*

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
IX (6 rats)	28	0	35.7
	36	0	35.3
	36	0	32.8
	48	9	37.0
	70	31	34.7
	Control 70	0	31.24
X (7 rats)	28	0	29.6
	33	0	30.7
	45	6	29.4
	45	6	31.6
	64	28	28.9
	72	32	27.6
	Control 72	0	25.1
XI (6 rats)	1	0	31.8
	27	0	28.6
	32	0	29.7
	53	18	25.8
	73	33	32.3
	Control 73	0	26.8

TABLE 9

*Bone ash determinations: deficiency diet from date of weaning; medication by aquasterol H (litter XIII) and cod liver oil (litter XII)*

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
XII (3 rats)	31	4	33.5
	31	4	33.6
	40	13	34.4
XIII (4 rats)	33	6	30.6
	40	13	29.7
	43	16	30.9
	52	25	27.6

# CONCLUSION

The practical conclusion to which these experiments point is that restoration of an approximately healthy growth area in the bones can be induced apart from replenishment of the skeletal mineral depots. A relatively minute water-soluble fraction obtained from cod liver oil or viosterol will remineralize and restore to a healthy appearance the growth area as effectively, though not necessarily with the same speed, as a relatively concentrated watery solution of crystalline activated ergosterol or as the oily preparations themselves. In bringing about this result vitamin D apparently utilizes mineral drawn from the shaft of the bones and possibly even from the body tissues.

Skeletal mineralization can be maintained only by an adequate mineral ration and an unimpaired mechanism of absorption and utilization. Skeletal demineralization will otherwise be progressive despite the administration of vitamin D.

# SUMMARY

1. The administration of aqueous preparations (aquasterol) of the antirachitic principle to growing white rats maintains or restores an approximately normal histological pattern in the growth area of bones despite severe pathological disturbance induced by a mineral-deficiency diet.

2. Accompanying this morphological restitution there is a maintenance of skeletal ash percentage in the animals receiving medication, provided that the velocity of growth is not out of proportion to the available mineral. Control animals not receiving medication show a progressive diminution in skeletal ash-content despite the fact that the mineral content of the diet is undiminished.

3. Histologically, commencing restoration of the normal pattern can be seen as early as the fourth day of medication; maximum restoration is evident on the ninth day. Continuation of the experiment upon the growing animal results in a progressive skeletal demineralization despite the maintenance of an approximately normal histological structure in the growth area.



4. It is therefore apparent that restitution of the normal morphology in the growth area and replenishment of skeletal mineral depots are two separate though usually closely related phenomena.

#### LITERATURE CITED

- BECCARI, EMILIO 1933 Azione e importanza terapeutica dei derivati solubili in acqua della vitamina D. *Arch. Internat. de Pharmacodynamie et de Thérapie*, vol. 44, pp. 164-172.
- BILLS, C. E., E. M. HONEYWELL, A. M. WIRICK AND M. NUSSMEIER 1931 A critique of the line test for vitamin D. *J. Biol. Chem.*, vol. 90, pp. 619-636.
- BOURDILLON, R. B., H. M. BRUCE, C. FISCHMANN AND T. A. WEBSTER 1931 The quantitative estimation of vitamin D by radiography. Medical Research Council. London. Special Report Series no. 158, pp. 1-46.
- DODDS, G. S. 1932 Osteoclasts and cartilage removal in endochondral ossification of certain mammals. *Am. J. Anat.*, vol. 50, pp. 97-127.
- DYER, F. J. 1931 The standardization of vitamin D by the line test. *Quart. J. Pharm. and Pharmacol.*, vol. 4, pp. 503-516.
- HUME, E. M., M. PICKERSGILL AND M. M. GAFFIKIN 1932 The determination of vitamin D. I. The relationship between graded doses of a standard solution of vitamin D administered to young rats on a rachitogenic diet and the ash content of their bones. *Biochem. J.*, vol. 26, pp. 488-505.
- MCCOLLUM, E. V., H. D. KRUSE AND E. R. ORENT 1933 The effects of deprivation of magnesium in the animal body. *Science*, vol. 77, p. 457.
- PALMER, L. S., AND C. KENNEDY 1931 The fundamental food requirements for the growth of the rat. VI. The influence of food consumption and the efficiency quotient of the animal. *J. Biol. Chem.*, vol. 90, pp. 545-564.
- SHIPLEY, P. C., E. A. PARK, E. V. MCCOLLUM, N. SIMMONDS AND H. T. PARSONS 1920-1921 Studies on experimental rickets. II. The effect of cod liver oil administered to rats with experimental rickets. *J. Biol. Chem.*, vol. 45, pp. 343-348.

# STUDIES ON THE ADRENAL

## VII. THE RELATION OF THE ADRENAL CORTICAL HORMONE TO THE VITAMINS <sup>1</sup>

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### FIVE FIGURES

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The idea of the existence of an intimate relationship between the adrenal cortex and certain vitamins (A, B (B<sub>1</sub>), C and G (B<sub>2</sub>)) has received increasing attention in recent years. This supposed relationship has been based upon: 1) the high concentrations of the vitamins (particularly A and C) occurring in the adrenal; 2) the changes occurring in the adrenal during avitaminosis (atrophy in avitaminosis A; hypertrophy in avitaminosis B and C); 3) the increase in susceptibility to infectious disease in avitaminosis and in adrenal insufficiency; 4) the similarity between certain of the clinical manifestations of avitaminosis and of adrenal insufficiency; 5) the alleged ameliorating effects of cortical extracts in avitaminosis B and C; 6) the similarity between certain of the chemical properties of the adrenal cortical hormone and vitamin G (B<sub>2</sub>).

Of the above considerations, the first may be dismissed as a coincidence due to solubility factors which determine the distribution of a given substance in the body. Thus the high

<sup>1</sup>The results recorded here were presented before the Federation of American Societies for Experimental Biology at New York, March, 1934.

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concentration of carotene (pro-vitamin A) in the cortex may be attributed to the high lipid content of the gland. Other lipoidal tissues, such as the corpus luteum (Huszek, '33) also contain equally high concentrations of pro-vitamin A, and we may therefore consider the existence of pro-vitamin A in the adrenal to be due to the laws of distribution based on simple solubility relationships. The adrenal atrophy occurring during avitaminosis A may be considered as part of a general reaction to the lack of a vital factor necessary for the well-being of many organs and tissues. There seems no reason, therefore, to assume an intimate relationship between vitamin A and the adrenal cortex, as Mitzkewitsch ('34) has also recently demonstrated.

In the case of vitamins B, C and G, however, the alleged ameliorating effects of adrenal cortical extracts in avitaminosis and similarities in the chemical properties of certain of these vitamins and the hormone, led us to reinvestigate the possible relationship between them.

#### VITAMIN B (B<sub>1</sub>)

Pico-Estrada ('27) demonstrated the increased susceptibility of adrenalectomized rats to avitaminosis B. It is well known, however, that adrenalectomized animals are hypersensitive to any abnormal condition—the administration of drugs, dietary deficiencies, temperature extremes, excitement, et cetera—and hence his observations indicate no special relationship between the adrenals and vitamin B.

Schmitz and Kühnau ('33) and Lockwood and Hartman ('33) have claimed that they could ameliorate the symptoms of vitamin B deficiency by administration of cortical extracts. However, since the adrenal cortex is rich in vitamin B content, it is imperative to remove all traces of this vitamin before one can conclude that the effects observed after administering a given extract are due to the cortical hormone rather than to simple contamination by the vitamin. The cortical hormone as prepared by most current methods is a relatively crude extract containing a number of constituents.

When one considers how difficult it is to remove the last traces of vitamin B from relatively pure casein or from refined

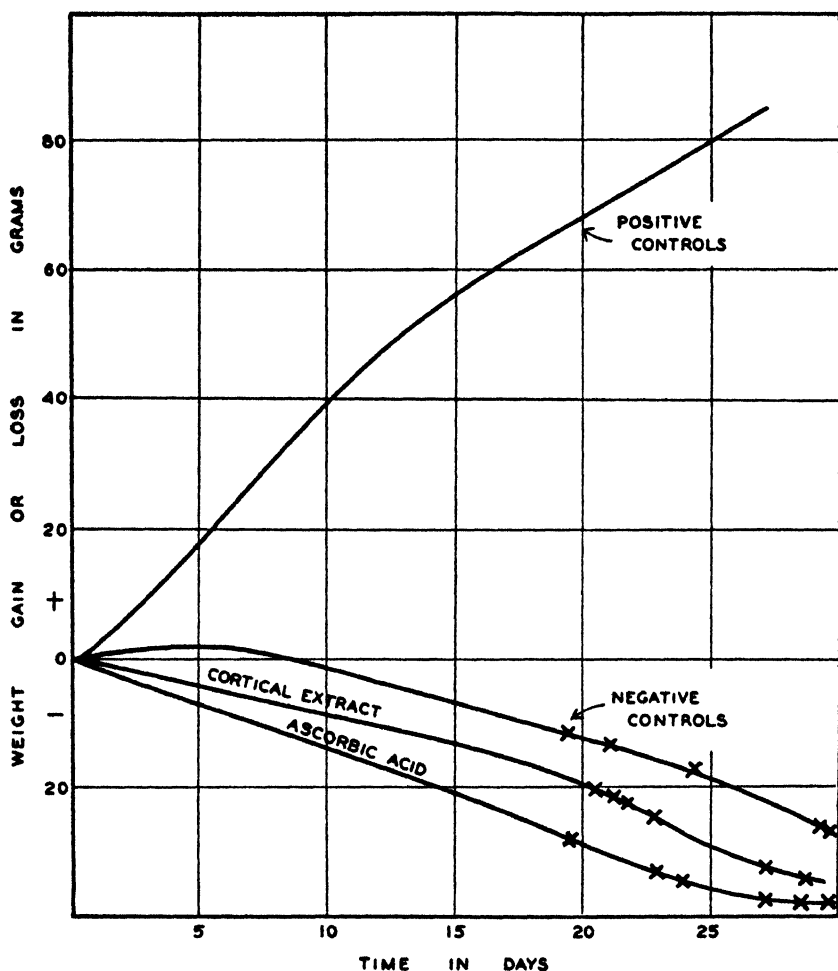


Fig.1 The effects of daily intraperitoneal injections of saline (negative controls), adrenal cortical extract and ascorbic acid on the weight curve and survival of rats on a vitamin B-free diet. X indicates the death of an animal. The abscissae indicate the days elapsing after the preliminary deprivation period.

lactose one might suspect the contamination by vitamin B of comparatively crude cortical extracts.

Our experiments (reproduced in fig. 1) were carried out on 4-week-old rats matched as to sex, weight and litter and

placed on Chase's modification of Sherman and Spohn's diet (Sherman and Smith, '31). All the precautions described by Sherman and Smith were carefully observed. The animals were divided into groups of seven. After the preliminary deprivation period of 2 weeks during which the animals assumed a constant weight, one group was maintained on the basal diet plus 10 per cent of whole ground wheat. These served as the positive controls. Another group received a daily intraperitoneal injection of 1 cc. of adrenal cortical extract (corresponding to 50 gm. of adrenal gland and assayed to contain two or more rat units) (Grollman and Firor, '34) prepared by the method of the authors ('33). Since cortical extracts are contaminated with variable amount of ascorbic acid (vitamin C), the effect of this substance on avitaminosis B was also determined on a third group of rats which received a daily intraperitoneal injection of 0.5 mg. of crystalline ascorbic acid<sup>3</sup> dissolved in 1 cc. of 0.8 per cent saline. A fourth group (negative controls) received a similar quantity of saline. The injections were continued for 30 days.

As seen in figure 1, the cortical extract had no ameliorating effects on the course of the avitaminosis. The group receiving ascorbic acid seemed to decline in weight much more rapidly and to develop more marked symptoms of avitaminosis than did the controls. This deleterious effect may be attributed to the stimulating action of an excess of vitamin C on metabolism, as has recently been demonstrated by Mosonyi and Rigo ('33). An excess of one vitamin may, thus, prove decidedly injurious to the organism.

The cortical extract used by us is relatively free of inert substances, and to this relative purity can be attributed our results. The opposite effects of Schmitz and Kühnau ('33) and Lockwood and Hartman ('33) must, on the other hand,

<sup>3</sup> A portion of the ascorbic acid used in this work was generously supplied by Prof. A. v. Szent-Györgyi to whom we wish to express our thanks. The remainder of the material was in part prepared by us by the method of Svirbely and Szent-Györgyi ('33) and in part obtained commercially. All samples were the pure crystalline product corresponding to the empirical formula  $C_6H_8O_6$  (Szent-Györgyi, '33).

be attributed to contamination by vitamin B derived from the original glandular material.

Considerable emphasis has been laid by previous writers (McCarrison, '21; Viale, '33) on the changes occurring in the adrenal in avitaminosis B. Gross and histological examination of the adrenals of our animals revealed no more striking changes than occurred in other endocrine glands and might have been anticipated from the inanition and retardation in growth accompanying vitamin B deficiency. In fatal cases of beriberi only minor changes in the adrenal have been noted (Hess, '20).

#### VITAMIN G (B<sub>2</sub>)

The tests with this vitamin were carried out on 4-week-old rats maintained on Bourquin and Sherman's diet (Sherman and Smith, '31)—purified casein, 18 per cent; Osborne-Mendel (Sherman and Smith, '31) salt mixture, 4 per cent; butter fat, 8 per cent; cod liver oil, 2 per cent and cornstarch 68 per cent. Vitamin B (B<sub>1</sub>) was supplied by adding to this mixture an alcoholic extract of whole wheat. After the preliminary depletion period, one group of animals (positive controls) were reared on the above diet to which was added 10 per cent of whole-milk powder. The other two groups of six animals were kept on the basal diet alone. One of these groups was given a daily intraperitoneal injection of 1 cc. of 0.8 per cent saline (negative controls), while the other received 1 cc. of cortical extract (derived from 50 gm. of whole adrenal glands) of the same potency as that used in the preceding experiments.

The animals used in the various groupings were litter mates matched for size and sex. All the precautions outlined by Sherman and Smith ('31) were rigidly observed.

The results of our experiment, reproduced in figure 2, show the absence of any effect of the cortical extracts in ameliorating the symptoms of avitaminosis G. Our cortical extracts are apparently free of vitamin G and the experiments of figure 2 show the absence of any beneficial effects of the adrenal cortical hormone in avitaminosis G.

## VITAMIN C

The fact that the adrenal cortex has such a high content of ascorbic acid has been adduced (Szent-Györgyi, '33; Viale, '33) as indicative of more than a casual relationship

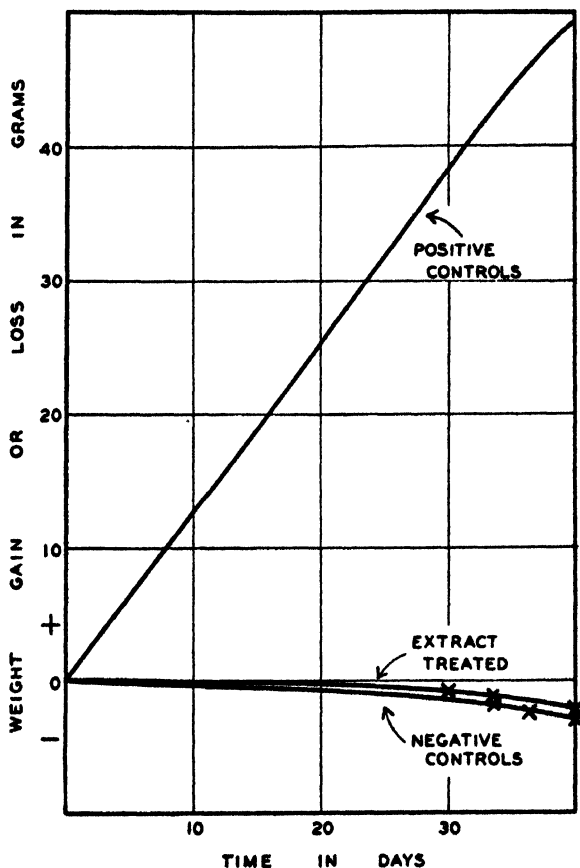


Fig. 2 The effects of daily intraperitoneal injections of adrenal cortical extract and saline (negative controls) on the weight curve and survival period of rats on a vitamin G-free diet. X indicates the death of an animal. The abscissae indicate the days elapsing after the preliminary deprivation period.

between the cortex and this vitamin. Grollman, Firor and Grollman ('34) have ascribed to this relationship a possible teleological significance for the close apposition of cortex and medulla in the adrenal.

The fact that vitamin C is present in such a high concentration in the adrenals renders it very difficult to prepare extracts of this gland which are free of the vitamin. The presence of ascorbic acid can be easily recognized in such extracts by spectrographic examination. In the cortical extracts prepared by the method of the authors ('33) relatively little ascorbic acid is present because of its elimination in the stage of preparation in which the cortical hormone is extracted from a neutral aqueous solution by benzol. The last traces of ascorbic acid may be easily removed by further purification. Such purified extracts were used in the following experiments. As a matter of fact, however, the amount of ascorbic acid present in our cortical extracts is less than that necessary to elicit any antiscorbutic activity, for essentially the same results were obtained when the unpurified cortical extract was used as were obtained after removal of the last traces of ascorbic acid. Spectrographic examination demonstrated the absence of ascorbic acid in our purified cortical extracts.<sup>4</sup>

In conducting our tests for any antiscorbutic activity of the adrenal cortical hormone 6- to 8-weeks-old guinea pigs, weighing 300 to 350 gm. were placed on the scorbutic diet of Sherman, La Mer and Campbell (Sherman and Smith, '31). This consisted of rolled oats, 39 per cent; bran, 20 per cent; skimmed milk powder, heated in open trays at 110°, 30 per cent; fresh butter fat, 10 per cent, and NaCl, 1 per cent. The weight curves of the animals were followed until death, when the animals were autopsied and examined for the typical manifestations of scurvy. The experiments were conducted with four groups of four animals in each experiment. One group was given the basal diet plus lettuce (positive controls); a second group was maintained on the basal diet plus a daily injection of 2 cc. of 0.8 per cent saline (negative controls); the third group was given an intraperitoneal injection of 2 cc. of extract (derived from 100 gm. of adrenal glands) daily.

<sup>4</sup> We wish to express our thanks to Dr. Sterling B. Hendricks for these spectrographic analyses.



The experiment was repeated three times (total of thirty-six animals), but inasmuch as the results were identical in each case, only a single experiment need be reproduced here (fig. 3).

As seen in figure 3 the daily administration of extract derived from 100 gm. of adrenal glands manifested no anti-scorbutic activity. This amount of extract corresponds roughly to several times that elaborated by the adrenal glands

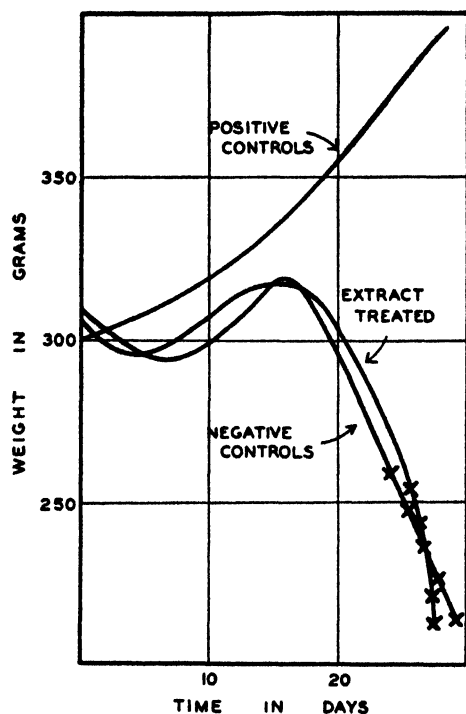


Fig. 3 The effects of daily intraperitoneal injections of adrenal cortical extract and saline (negative controls) on the weight curve and survival of guinea pigs on a scorbutic diet. X indicates the death of an animal.

of the normal guinea pig, as judged from its life-prolonging action in adrenalectomized animals, and should have been ample, therefore, to elicit any antiscorbutic activity. Autopsy of our experimental animals revealed the same degree of scurvy in our extract-treated animals (average scurvy score, 20) as in the negative controls (average scurvy score, 21) (Hess, '20; Sherman and Smith, '31).

Barbieri and Gambaro (quoted by Viale, '33) claimed that they could prolong the life of guinea pigs on a vitamin C-free diet with cortical extracts. Lockwood and Hartman ('33) in an extended study found that intraperitoneal injection of 'cortin' improved the growth curve in avitaminosis B and C, and ameliorated the symptoms when they occurred. Their results must be attributed to contamination of their extracts with ascorbic acid rather than to any effect of the cortical hormone itself, as they inferred.

In support of the view that the beneficial effects of 'cortin' in avitaminosis are not due to an admixture of ascorbic acid in their extracts, Lockwood and Hartman ('33) have advanced two arguments: 1) the insolubility of ascorbic acid in ether, which they used as their extraction medium, and, 2) the ineffectiveness of 'cortin' when administered orally. Their first argument can carry little weight, however, for a given solvent will, as is well known, extract substances from a gland which in a pure state would be insoluble in the solvent. Moreover, although relatively insoluble in ether the large volumes of the solvent used by these authors would dissolve an appreciable amount of ascorbic acid even in the pure form. Their second argument is based on another fallacy, namely, the assumption that the efficacy of ascorbic acid when administered orally is equal to that of the same amount of the substance injected parenterally. As a matter of fact, a priori considerations based on the chemical properties of ascorbic acid would lead one to suspect that its oral administration would require, as is the case with many therapeutic agents, larger doses than does intraperitoneal administration. Hence, the injection of a very small dose (present as an admixture in crude adrenal extracts) intraperitoneally might lead to an amelioration of the scorbutic symptoms which would not be detected after oral administration. The experimental demonstration of the correctness of this view is reproduced in figure 4.

Groups of twelve guinea pigs were maintained on the scorbutic diet described above. Besides the usual positive (lettuce

added to diet) and negative controls, one group was injected daily with  $\frac{1}{4}$  mg. of ascorbic acid dissolved in  $\frac{1}{2}$  cc. of 0.8 per cent saline, while the second received an equal quantity of the vitamin, orally. The curves of figure 4 show the greater therapeutic activity of the parenterally administered hormone. Whereas all of the animals treated orally died during

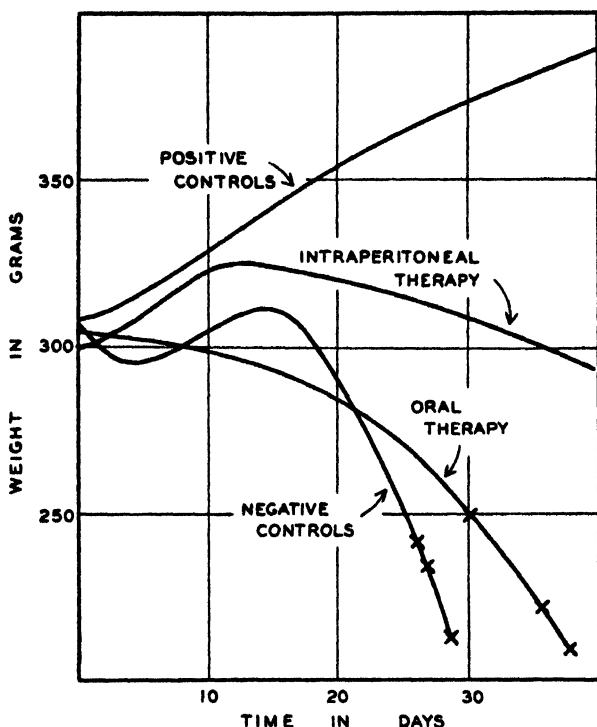


Fig. 4 A comparison of the effects of the oral and intraperitoneal administration of  $\frac{1}{4}$  mg. of crystalline ascorbic acid daily on the weight curve of guinea pigs on a scorbutic diet. X indicates the death of an animal.

the experimental period with marked scurvy (average scurvy score, 18); those injected intraperitoneally with the same amount of material showed prolonged survival and only minor degrees of scurvy at autopsy (average scurvy score, 9). This is essentially the same result obtained by Lockwood and Hartman in their study of the comparative effects of the oral and intraperitoneal administration of cortical extract and we must

conclude that their results are due to the relative efficacy of different modes of administration. Their extracts apparently contained about  $\frac{1}{4}$  mg. of ascorbic acid in 2 cc. (their dosage) which, as we have seen, is not unexpected.

Considerable emphasis has recently been placed on the hypertrophy and abnormalities occurring in the adrenal during avitaminosis C. It is questionable how much of the observed hypertrophy is due to the starvation accompanying scurvy and to concurrent infections, which are common, and both of which, as Hess ('20) has pointed out, complicate the picture. It is significant that in human scurvy there are no marked alterations in the adrenal (Hess, '20). In our own animals we have frequently observed hemorrhage of the adrenals similar to that which is found in the muscles, gastrointestinal tract, gums, et cetera, and which, therefore, is not a specifically adrenal manifestation. The medulla often presents a grossly altered appearance being converted to a soft, chocolate-colored, hemorrhagic mass.

#### THE EFFECT OF ASCORBIC ACID IN ADRENALECTOMIZED RATS

Because of the close association between ascorbic acid (vitamin C) and the adrenal cortex, it seemed desirable to test the effects of this substance on adrenalectomized rats. The results of a typical experiment are given in figure 5. The upper curve shows the effect of administering the cortical extract prepared as described by us (Grollman and Fitor, '33) to bilaterally adrenalectomized rats. When the animals are treated with a potent extract as in the upper curve of figure 5, they grow normally<sup>5</sup> until several days after cessation of treatment, after which they rapidly lose weight and die. Untreated controls, as shown in the lowest curve, fail to gain weight and die in a much shorter period of time than the treated animals.

<sup>5</sup> The results of Gaunt and Gaunt ('34), which led them to question the validity of the rat method for the assay of the adrenal cortical hormone, are due to faulty operative technic, as previous workers and we (Grollman and Fitor, '34) have demonstrated. The results of figure 5 illustrate the adequacy of our method of assay.

As seen in the middle curve of figure 5, the daily administration of 1 mg. of ascorbic acid intraperitoneally has no demonstrable effect on the survival or weight curve of adrenalectomized rats. This observation is in accord with the statement of Szent-Györgyi ('33) that ascorbic acid has no beneficial effect in patients suffering from Addison's disease. This observer states, however, that administration of vita-

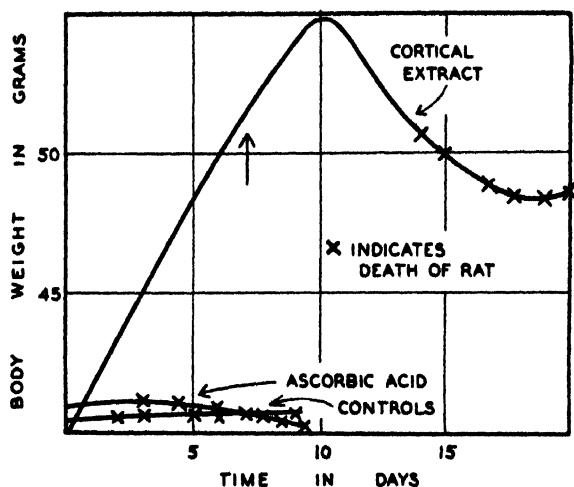


Fig. 5 An assay illustrating the absence of any effect of ascorbic acid on the weight curve and survival of adrenalectomized rats. The upper curve shows the response of the operated animals to the administration of adrenal cortical extracts for 7 days. The lowest curve (controls) shows the response to saline injections. All operations were performed at one sitting under amytal anesthesia. X indicates the death of an animal. The arrow indicates the day on which the injections were discontinued.

min C removes the bronzing of such patients. Accordingly, we must consider this important clinical feature of Addison's disease as being only a secondary result of disease of the adrenal cortex and not due to insufficiency of the cortical hormone itself. The occurrence of the typical clinical picture of Addison's disease without pigmentation (Ghrist and Rowntree, '27) may be explained on the basis of the above considerations.

## DISCUSSION

The above experiments are evidence against the view expressed by many previous authors that there exists a particularly intimate relation between the vitamins and the adrenals. The susceptibility of the adrenal gland to conditions of avitaminosis is not unique, for other endocrine organs (thyroid, pituitary, sex glands, etc.) are equally affected (McCarri-son, '21) by dietary deficiencies. The vitamins, apparently, are essential for the well-being of a great number, if not all, of the organs and tissues of the body. The similarity between certain clinical manifestations of dietary deficiencies and of adrenal insufficiency are due to the fact that the cortical hormone is also necessary for the well-being of a number of tissues and organs. Hence, deficiency due to either vitamin or hormone leads to a multiplicity of manifestations (in the gastro-intestinal, nervous, cardiovascular, endocrine and other systems) and to a superficially similar symptomatology. The increased susceptibility to infectious disease in avitaminosis and in adrenal insufficiency is only an indication of a general lowering of body-resistance—a condition occasioned by a number of causes.

## SUMMARY

The adrenal cortical hormone was found to have no demonstrable replacement activity in experimental avitaminosis B or G.

The apparent partial replaceability of vitamin C by adrenal cortical preparations reported by previous observers is due to the presence of ascorbic acid in such extracts. Crystalline vitamin C is more effective when administered intraperitoneally than by mouth. Ascorbic acid does not prolong the life of adrenalectomized animals.

The other considerations which have led previous observers to assume the existence of an intimate relation between the adrenal cortex and vitamin deficiencies are explicable on the assumption that the hormone and vitamins are necessary for the proper functioning of a great number of organs and tissues.

## LITERATURE CITED

- GAUNT, R., AND J. H. GAUNT 1934 Survival of adrenalectomized rats after cortical hormone treatment. *Proc. Soc. Exp. Biol. and Med.*, vol. 31, p. 490.
- GHEIST, D. G., AND L. G. ROWNTREE 1927 Addison's disease without pigmentation. Report of a case. *Endocrinology*, vol. 11, p. 589.
- GROLLMAN, A., AND W. M. FIBOR 1933 Studies on the adrenal. III. The preparation of an active extract of the hormone of the adrenal cortex. *J. Biol. Chem.*, vol. 100, p. 429.
- 1934 Studies on the adrenal. IV. The oral administration of the adrenal cortical hormone and the use of fresh glands therapeutically. *Bull. Johns Hopkins Hosp.*, vol. 54, p. 216.
- GROLLMAN, A., W. M. FIBOR, AND E. GROLLMAN 1934 The extraction of the adrenal cortical hormone from the interrenal body of fishes. *Am. J. Physiol.*, vol. 108, p. 237.
- HESS, A. F. 1920 Scurvy, past and present. Lippincott & Co., Philadelphia.
- HUSZAK, S. 1933 Ueber den Ascorbinsäuregehalt der Corpora lutea. *Zeit. f. physiol. Chem.*, Bd. 219, S. 275.
- LOCKWOOD, J. E., AND F. A. HARTMAN 1933 Relation of the adrenal cortex to vitamins A, B<sub>1</sub> and C. *Endocrinology*, vol. 17, p. 501.
- MCCARRISON, R. 1921 Studies in deficiency disease. Oxford University Press, London.
- MITZKEWITSCH, M. S. 1934 Der Zustand der endokrinen Drüsen während der A-Avitaminose bei Ratten. *Arch. f. exp. Path. u. Pharm.*, Bd. 174, S. 339.
- MOSONYI, J., AND L. RIGO 1933 Ueber den Einfluss des Vitamins C auf den Stoffwechsels normaler und skorbutkranken Meerschweinchen. *Zeit. f. physiol. Chem.*, Bd. 222, S. 100.
- PICO-ESTRADA, O. 1927 Sensibilité des rats privés de surrénales à l'avitaminose B. *Compt. rend. de la soc. de biol.*, T. 97, p. 1031.
- SCHMITZ, E., AND J. KÜHNAU 1933 Ueber die innere Sekretion der Nebennierenrinde. *Biochem. Zeit.*, Bd. 259, S. 301.
- SHERMAN, H. C., AND S. L. SMITH 1931 The vitamins. The Chemical Catalogue Co., New York.
- SVIRBELY, J. L., AND A. SZENT-GYÖRGYI 1933 The chemical nature of vitamin C. *Biochem. J.*, vol. 27, p. 279.
- SZENT-GYÖRGYI, A. 1933 L'acide ascorbique (vitamine C). *Bull. de la soc. de chim. biol.*, T. 15, p. 694.
- VIALE, G. 1933 L'ormone cortico-surrenale. *Monitore di endocrinol. per il med. pratico.*, T. 1, p. 100.

# THE EFFECT OF SUPPLEMENTARY IODINE ON THE NUTRITIVE VALUE OF CHICK RATIONS

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## FOUR FIGURES

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As early as 1851, Chatin (1851) observed that the prevalence of goiter varied with the iodine content of the soil of different localities. The subsequent extensive studies of the factors which influence the development of human goiter confirm this observation. Furthermore, chemical analyses of numerous fruits, vegetables, milk, etc., by Forbes and co-workers ('16), McClendon and associates ('30), Remington ('30) and others show a wide variation in iodine content depending upon the iodine content of the soils upon which the materials were produced.

Numerous investigators have shown that a continued deficiency of iodine in the rations of farm animals produces various abnormal conditions. Welch ('28) observed goiter in foals, calves, lambs, pigs, goats and dogs. He says, "Goiter of the adult animal, as compared with goiter of the new-born, is relatively rare." He also states that the loss of young pigs "was so great that swine breeding was practically abandoned in many sections of Eastern Montana and Western North Dakota," but the use of iodine as a prophylactic agent eliminated the difficulty. Shepperd ('29) and others have associated deficiency of iodine intake with the hairless pig malady. Fenger and associates ('31) found the iodine content of the thyroid gland (hog) varied with the geographical



location from which the animals came. Kalkus ('20) says, "It is variously estimated by stockmen that from 75 to 90 per cent of the colts in some of these goiterous districts are affected with the trouble, and that 95 per cent of such animals are either still-born or die two or three days later. The remaining 5 per cent never make perfect recovery." Hudson ('33), Harvey ('29), Cooley ('31), Rodenwold and Simms ('34) and others have reduced the prevalence of navel disease or joint ill in newborn foals by feeding potassium iodide to mares during pregnancy. Several investigators have reported that administration of iodine influences reproduction in farm animals. For instance, Phillips, Curtis and Erf ('34) say "cows of the herd previously sterile have become pregnant and borne normal calves during this regimen of feeding." Marine and Kimball ('21) state that sodium iodide has been successfully used on a large scale in the prevention of goiter in cattle, sheep, pigs and poultry. Weiser and Véghelyi ('32) report that merino sheep, of an iodine deficient region, when fed iodine regularly produced larger lambs and 10 per cent more wool than a control flock.

These varied observations concerning the effect of an inadequate supply of iodine on the health of hogs, horses, cattle and sheep naturally raise a question as to whether commercial poultry rations contain an adequate supply of iodine. The cereal grains which constitute a larger portion of commercial poultry rations are grown on soils far removed from the ocean and of reputed low iodine content. Numerous analyses by Forbes and co-workers ('32) show that corn, oats and wheat rarely contain more than a 'trace' of iodine and the majority of the samples contained 'none.' Welch ('28) reports "Goiter in poultry is very common. Flocks with a very high percentage of it have been noted in goiterous areas." On the other hand, Kernkamp ('25), who has made a detailed report of simple colloid goiter in poultry states that goiter in poultry is very uncommon.

Hercus and Roberts ('27), Simpson and Strand ('30), Jaschik and Kieselbach ('31), Scharrer and Schropp ('32),

Schmidt ('32), Zickgraf ('32), Straub ('33), and Wilder, Bethke and Record ('33) increased the iodine content of eggs by supplementing the ration of laying hens with various iodine compounds. Also Scharrer and Schropp ('32) and Klein ('33) found that supplemental iodine increased egg production, but Malan ('31) obtained the same egg yield for both experimental and control birds. The results that were observed when the rations of adult birds were supplemented with iodine suggested the possibility of enhancing the nutritive value of rations for growing chicks by supplementing them with iodine compounds. The present investigation was conducted to obtain data on this point, but no attempt was made to accumulate data concerning the effects of iodine deficiency or concerning the iodine requirement of growing chicks.

#### EXPERIMENTAL

The experimental mashes were prepared on a commercial scale in a thoroughly modern feed manufacturing plant. The basal mash was designed to include an adequate amount and a satisfactory proportion of the constituents essential for rapid, balanced growth of young chicks. It contained corn meal (attrition) 32 per cent, wheat bran 15 per cent, wheat flour middlings 15 per cent, ground oat groats 12 per cent, alfalfa leaf meal 5 per cent, dry skim milk 8 per cent, meat scraps 5 per cent, fish meal 5 per cent, sardine oil 0.5 per cent, dicalcium phosphate 0.5 per cent, oyster shell meal 1 per cent and salt 1 per cent. On analysis this ration was found to contain: moisture 9.75 per cent, protein 19.35 per cent, fat 5.50 per cent, fiber 4.30 per cent, carbohydrates 57.35 per cent, ash 9.10 per cent, calcium 1.57 per cent, phosphorus 1.03 per cent and approximately 500 parts of iodine per billion parts of mash. The basal mash was divided into five lots. One lot was retained as 'control' mash and potassium iodide was added to the other lots at the rate of 18.8 mg., 37.5 mg., 75.0 mg. and 93.8 mg. per kilogram of mash. The potassium iodide was first mixed with the salt. These were combined with the other mineral constituents of the ration and this

in turn was carefully incorporated into the ration. The mashes under consideration were assigned as follows:

Pen A—Basal mash

Pen B—Basal mash plus 18.8 mg. KI per kilogram

Pen C—Basal mash plus 37.5 mg. KI per kilogram

Pen D—Basal mash plus 75.0 mg. KI per kilogram

Pen E—Basal mash plus 93.8 mg. KI per kilogram

Rhode Island Red baby chicks purchased from a hatchery which maintained high producing, bacillary white diarrhea free stock served as subjects of this investigation. The parent stock was fed a combination of 'laying mash' and scratch grains. Data concerning the iodine intake of the parent stock are not available. Analysis of 'laying mash' manufactured in the same mill and composed of similar ingredients showed an iodine content of 604 parts per billion. Practical experience indicates that the amount of iodine supplied by a combination of this type of 'laying mash' and scratch grains meets the requirements of breeding stock. More definite information in this connection would be of interest, since Corrie ('33) reports that iodine deficiency in the ration of the laying hen causes a lowered resistance to disease in the progeny, and Weiser and Zaitschek ('32) state that the development of pigs is advantageously influenced by feeding iodine to pregnant sows. On the other hand, Scharrer and Schropp ('32) compared the results obtained in two groups of laying White Leghorn hens, one of which received KI as a supplement to its ration, and found "Growth of the chicks was equally good in both groups."

In general, the experimental procedure was similar to that employed in previous studies (Holmes et al., '33). The chicks were housed in all-metal battery brooders equipped with automatically controlled, electrically heated hovers. The windows of the battery room were covered and throughout the experiment all light was obtained from two 200-watt ceiling lamps which were surrounded with shields known as Red Laco Color Lights. The chicks were divided into five comparable groups of thirty chicks. From hatching until 12 weeks

of age the chicks received only the experimental mashes and drinking water, both of which were continuously before the chicks throughout the entire experimental period.

The chicks were weighed once a week. The average per chick weights for the cockerels and pullets are reported as

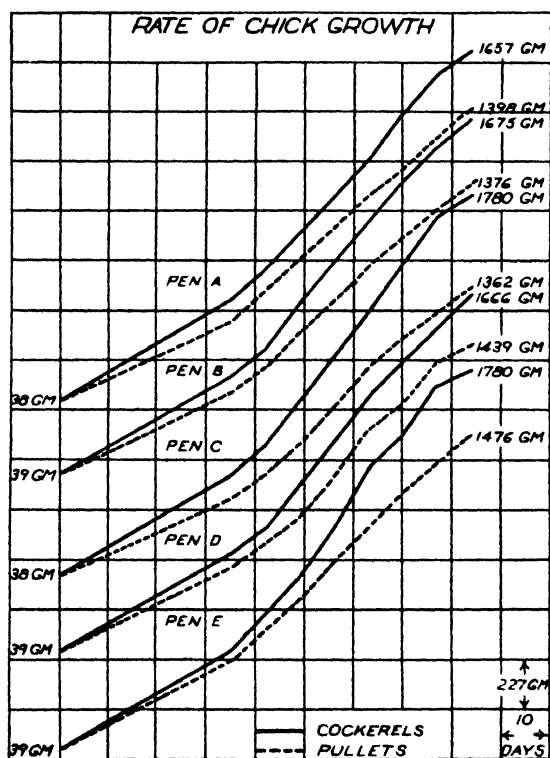


Fig. 1 The figures at the left of the growth curves indicate the initial weight of the baby chicks. The figures at the right indicate average per chick weight for cockerels and pullets at the termination of the experiment when the chicks were 12 weeks old.

growth curves in figure 1. The rate of growth of the chicks in the different pens was quite uniform and consistent during the experimental period. At 12 weeks of age the weight of the cockerels was 1657, 1675, 1780, 1666 and 1780 gm. and that of the pullets was 1398, 1376, 1362, 1439 and 1476 gm., respectively, for pens A, B, C, D and E. These weights show

that the growth of the chicks in all the pens was excellent. The weights of the cockerels of pens C and E are greater than that of the control pen. Likewise the weights of the pullets of pens D and E are higher than that of the control pen. On the other hand, the weight of the pullets in pens B and C, which received iodine supplements, is less than that of the control pen. These data do not indicate that supplementary iodine consistently enhances the growth promoting value of the basal ration used in this study.

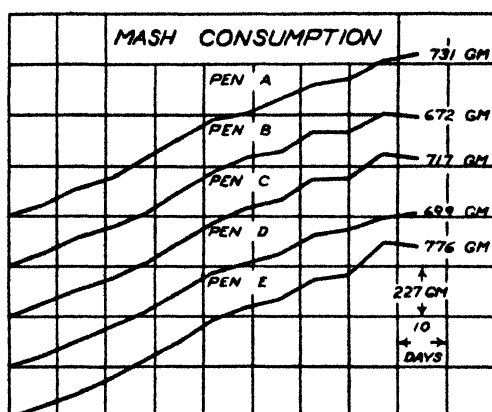


Fig. 2 The figures at the right of the curves indicate the average per chick mash consumption for the final (twelfth) week of the experiment.

The amount of mash eaten by each of the experimental pens was determined each week and this was reduced to a per chick per week basis. The mash consumption curves for the various experimental pens appear in figure 2. From these it will be noted that the amount of mash consumed per bird increased consistently and quite uniformly during the entire experimental period. The total mash consumed per bird for the entire experimental period was 5080, 4920, 5080, 4920 and 5260 gm. for pens A, B, C, D and E, respectively. Inasmuch as pen E, which received the highest amount of iodine, consumed the largest amount of mash it is evident that the continued ingestion of this amount of iodine did not decrease its palatability.

The nutritive efficiency of the five mashes under consideration has been computed on the basis of the amount of mash required to produce 100 gm. gain in weight. Such computations have been made at the close of each week of the test period. These data for the five mashes have been assembled as curves which appear in figure 3. The figures which appear

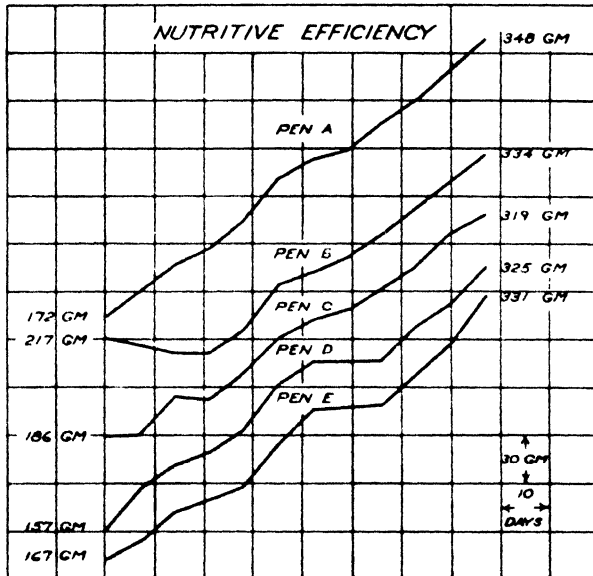


Fig. 3 The figures at the left of the curves indicate the amount of mash required to produce 100 gm. gain in body weight during the first week and those at the right indicate the amount required per 100 gm. of gain in weight for the entire 12-week period. The least amount of mash required to produce 100 gm. gain in weight was for pen C, hence mash C had the highest nutritive efficiency.

at the right of the curves represent the efficiency of the mashes for the entire 12-week period. Since the efficiency of the mashes is in inverse ratio to the amount consumed, mash C, which required 319 gm. to produce 100 gm. gain in weight, was the most efficient and mash A, which required 348 gm. to produce 100 gm. gain, was least efficient. If attention were confined to the results obtained with pens A, B and C, one would conclude that the nutritive efficiency of the control mash

increased as the amount of supplementary iodine was increased. However, the amount of iodine contained in ration D is twice that of ration C and yet the nutritive efficiency is lower. In the same manner mash E has five times the supplementary iodine present in mash B and the nutritive efficiency is essentially the same.

The physical appearance of the chicks (every bird) was carefully observed each week of the experiment. Attention was directed to the extent of feathering, the number of pinfeathers, the color of the shanks and beaks, the extent of development and the color of comb and wattles, and the general uniformity and alertness of the groups as a whole. Judged by these characteristics there was no significant difference in the physical appearance of the birds of the five pens under consideration.

The bone development of the chicks was judged by observations made on tibiae removed from five typical test chicks of each pen at the end of the 12-week period. The left tibiae were freed of tissue as soon as they had been removed from the birds, dried at 100°C. for 48 hours. They were then measured and weighed individually. These data were averaged for each pen. The dried left tibiae were ground and extracted with 95 per cent alcohol for 24 hours. For this purpose the entire tibia including the cartilage at the proximal end was used. The combined, dried, extracted tibiae of the five test chicks were incinerated to determine the ash content. The data for length, diameter, weight and ash content of the tibiae will be found in table 1. The length and diameter of the tibiae was least for the control pen and greatest for pen E, which received the largest amount of iodine. On the other hand, the average weight per tibia was greatest for pen B, which received the least amount of supplementary KI, and the tibia ash content was highest for pen C, which received one of the lower levels of KI. In view of these inconsistent results for the length, diameter and weight of the tibiae and for their ash content, it is questionable whether the addition of KI significantly improved the bone building value of the control ration.

When the right tibiae were removed from the test chicks they were carefully freed of all tissue and placed in formaldehyde for a number of days. Subsequently they were split, washed, stained with a 2 per cent silver nitrate solution, intensified under a bright light and photographed. The accompanying photograph (fig. 4) of a section of a tibia from a typical chick of each pen shows the internal structure of the tibiae from chicks which received the basal ration and the basal ration supplemented with different amounts of potassium iodide. Examination under a binocular microscope (thirteen diameters) of the tibiae sections from the five chicks of each experimental pen did not reveal any difference in the development of the tibiae of chicks which received supplementary KI from that of the control chicks.

TABLE 1  
*The tibia growth of experimental chicks*  
(12-week-old chicks)

PEN	LENGTH	DIAMETER	WEIGHT	ASH
	<i>cm.</i>	<i>cm.</i>	<i>gm.</i>	<i>Per cent</i>
A	11.67	0.75	8.11	46.89
B	11.93	0.90	9.01	47.40
C	12.00	0.88	8.71	48.85
D	12.14	0.88	8.33	48.11
E	12.30	0.95	8.91	47.30

Hemoglobin determinations were made of the blood of typical chicks, three cockerels and four pullets from each pen, at the termination of the experimental period. The determinations were made by the procedure previously described (Holmes et al., '33). The results obtained are reported in table 2. It will be noted that the hemoglobin content of the blood was highest for the control pen, pen A. While the hemoglobin values for the chicks which received supplementary iodine increased with the amount of iodine fed, they did not equal that of the control pen. These data show that the use of supplementary iodine did not increase the hematopoietic value of the basal ration. In fact, one might conclude that it tended to decrease the hematopoietic value of the control mash.



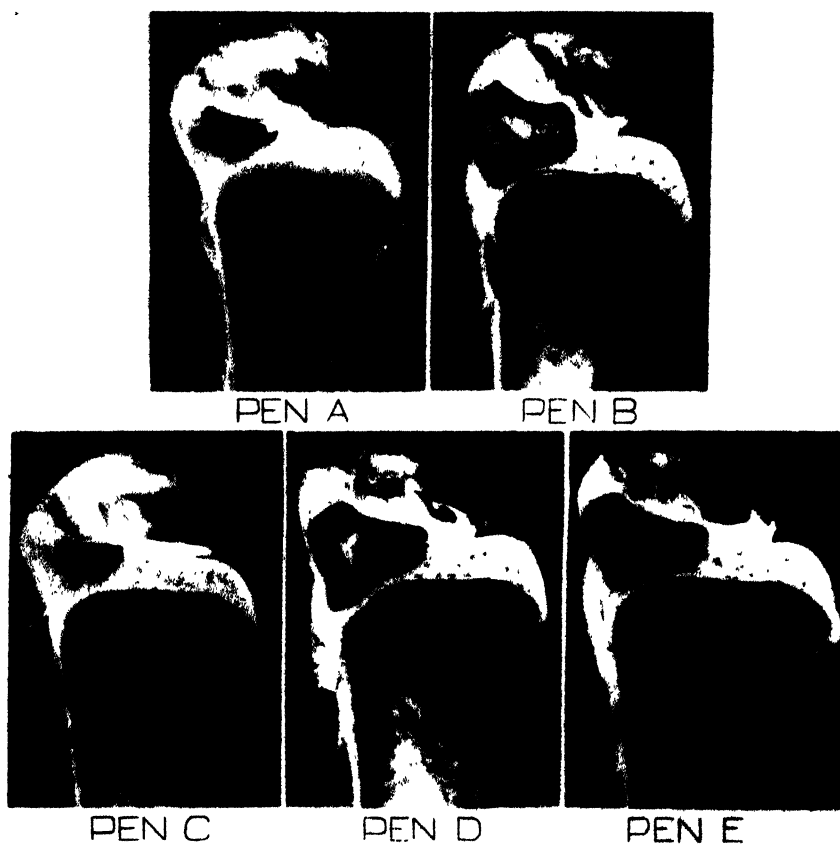


Fig. 4 Photographs of sections of split right tibiae of 12-week-old chicks. No significant difference can be detected in the extent of calcification of the tibiae of chicks from the different pens.

TABLE 2  
*Hemoglobin content of chicken blood*  
(12-week-old chicks)

PEN	COCKERELS	PULLETS	AVERAGE <sup>1</sup>
	(Gm. Hb. per 100 cc. of blood)		
A	10.37	10.03	10.24
B	9.02	9.45	9.26
C	9.22	9.54	9.37
D	10.04	9.93	9.96
E	9.96	10.31	10.16

<sup>1</sup> Weighted average.

The data which were obtained in this investigation are in accord with those reported by other investigators. In a carefully controlled experiment conducted by Hamilton and Kick ('30) "no evidence was obtained that a supplement of potassium iodide at the rate of 0.5 mg. or of 1.0 mg. daily per 100 gm. of body weight in any way influenced the rate of growth." Forbes and associates ('32) studied the influence of iodine on the growth of White Leghorn pullets (701). Groups 1 and 2 received the same mash and groups 3 and 4 another mash of different nutritive value. Groups 2 and 3 also received a sufficient amount of iodized linseed meal "to provide 50 mg. of iodine per 100 pounds of chicken a day." When 12 weeks old, the chicks of the control groups weighed more than the corresponding groups which received the iodine supplement to their ration.

#### SUMMARY

Five pens of thirty Rhode Island Red chicks were fed a high quality chick growing mash from hatching to 12 weeks of age under standardized laboratory conditions. The mash for four pens was supplemented with 18.8 mg., 37.5 mg., 75.0 mg. and 93.8 mg. of KI per kilogram of mash.

Observations were made on the growth, physical appearance and mash consumption of the chicks; the nutritive efficiency of the mashes; the bone growth and the hemoglobin content of the blood when the chicks were 12 weeks of age.

The results obtained were somewhat irregular. The final weight of the chicks did not vary consistently with the amount of iodine fed. The mash consumption was greatest for the pen receiving the most KI. The physical appearance was similar for all pens. The nutritive efficiency for mash C, to which was added 37.5 mg. of KI, was higher than that of the mashes which contained more or less KI. The length and diameter was greatest for tibiae from the chicks that received the most KI. On the other hand, the heaviest tibiae were from pen B and the ash content was larger for chicks from pen C than for those chicks which received more or

less KI. The internal structure of the tibiae showed the same degree of development for all pens. The hemoglobin content of the blood of the 12-week-old chicks was greatest for the control pen.

The variations in the results obtained for the different experimental pens with respect to growth, mash consumption, physical appearance of the birds, nutritive efficiency of the mash, the size and composition of the bones, the internal structure of the tibiae, and the hemoglobin content of the blood do not appear to be highly significant, since these variations are not materially larger than might occur for two 'control' pens maintained under comparable conditions.

These results show that the amounts of KI fed as a supplement to the control ration did not significantly enhance its nutritive value. Since the chicks which received the control ration grew exceptionally well and showed satisfactory development in all respects, it would appear that the control ration contained an adequate supply of iodine to meet the needs of rapidly growing chicks.

#### LITERATURE CITED

- CHATIN, A. 1851 Recherche de l'iode dans l'air, les eaux, le sol et les produits alimentaires des Alpes, de la France et du Piemont. *Compt. rend.*, T. 33, p. 529.
- COOLEY, R. B. 1931 Mineral deficiencies in rations for horses. *Purdue Univ. Animal Husbandry Seminar*, January 23.
- CORRIE, F. E. 1933 Mineral supplement to farm foods. *Chemist and Druggist*, vol. 119, p. 444.
- FENGER, F., R. H. ANDREW, AND J. J. VOLLERTSEN 1931 Geographic location and the iodine content of the thyroid gland. *J. Am. Chem. Soc.*, vol. 53, p. 237.
- FORBES, E. B., F. M. BEEGLE, C. M. FRITZ, L. E. MORGAN, AND S. N. RHUF 1916 The iodine content of foods. *Ohio Agr. Exp. Sta. Bull.* 299, June.
- FORBES, E. B., G. M. KARNS, S. I. BECHDEL, P. S. WILLIAMS, T. B. KEITH, E. W. CALLENBACH, AND R. R. MURPHY 1932 The value of iodine for livestock in central Pennsylvania. *J. Agr. Res.*, vol. 45, p. 111.
- HAMILTON, T. S., AND C. H. KICK 1930 The value of potassium iodide as a supplement to the ration of growing chicks. *J. Agr. Res.*, vol. 41, p. 135.
- HARVEY, A. L. 1929 Feeding potassium iodide to mares. *Proc. Am. Soc. Animal Prod.*, p. 93.

- HERCUS, C. E., AND K. C. ROBERTS 1927 The iodine content of foods, fertilizers and animal products in relation to the prophylaxes of endemic goiter in New Zealand. *J. Hygiene*, vol. 26, p. 49.
- HOLMES, A. D., M. G. PIGOTT, AND P. A. CAMPBELL 1933 The hemoglobin content of chicken blood. *J. Biol. Chem.*, vol. 103, p. 657.
- HUDSON, R. S. 1933 The mare and foal. *Mich. State College Extension, Bull.* 128.
- JASCHIK, A., AND J. KIESELBACH 1931 Iodized eggs. *J. Ztschr. f. Untersuch. d. Lebensmittel.* Bd. 62, S. 572.
- KALKUS, J. W. 1920 A study of goiter and associated conditions in domestic animals. *Wash. Agr. Exp. Sta. Bull.* 156.
- KERNKAMP, H. C. H. 1925 Goiter in poultry. *J. Am. Vet. Med. Assoc.*, vol. 67, p. 223.
- KLEIN, W. 1933 Addition of iodine to poultry feeds. Effect of iodine on egg yield of two year old hens before, during and after molting. *Arch. f. Geflügelk.*, Bd. 7, S. 65.
- MALAN, A. I. 1931 Studies in mineral metabolism. 15. Potassium iodide in poultry farming. 17th Rep. Director Vet. Services and Animal Ind., Union of S. Africa, p. 439.
- MARINE, D., AND O. P. KIMBALL 1921 The prevention of simple goiter in man. *J. Am. Med. Assoc.*, vol. 77, p. 1068.
- McLENDON, J. F., R. E. REMINGTON, H. VON KOLNITZ, AND R. RUFÉ 1930 The determination of traces of iodine. III. Iodine in milk, butter, oil and urine. *J. Am. Chem. Soc.*, vol. 52, p. 541.
- PHILLIPS, F. J., G. M. CURTIS, AND O. ERF 1934 Results of prolonged increased iodine feeding. *Proc. Soc. Exp. Biol. and Med.*, vol. 31, p. 585.
- REMINGTON, R. E. 1930 Unanswered questions in the biochemistry and geochemistry of iodine. *J. Chem. Education*, vol. 7, p. 2590.
- RODENWOLD, B. W., AND B. T. SIMMS 1934 Goiter in foals. *Percheron News*.
- SCHARRER, K., AND W. SCHROPP 1932 Experiment in feeding iodine to hens. *Biedermanns Zentr. B. Tierernähr.*, Bd. 4, S. 249.
- SCHMIDT, J. 1932 Feeding of iodine to poultry. *Berl. tierärzt. Wochenschr.*, Bd. 48, S. 161.
- SHEPPERD, J. H. 1929 The Northern pig, its habits, breeding and management. *N. D. Agr. Exp. Sta. Bull.* 230, July.
- SIMPSON, B. W., AND R. STRAND 1930 Feeding iodine to poultry. *New Zealand J. Agr.*, vol. 40, p. 403.
- STRAUB, J. 1933 Iodine content of iodine eggs. *Z. Untersuch. Lebensm.*, Bd. 65, S. 97.
- WEISER, S., AND E. VÉGHÉLYI 1932 Iodine tolerance in sheep and lambs. *Fortchr. d. Landwirtschaft.*, Bd. 7, S. 289.
- WEISER, I., AND A. ZAITSCHEK 1932 The iodine tolerance of swine. *Biedermanns Zentr. B. Tierernähr.*, Bd. 4, S. 476.
- WELCH, H. 1928 Goiter in farm animals. *Mont. Agr. Exp. Sta. Bull.* 214, June.
- WILDER, O. H. M., R. M. BETHKE, AND P. R. RECORD 1933 The iodine content of hens' eggs as affected by the ration. *J. Nutrition*, vol. 6, p. 407.
- ZICKGRAF, G. 1932 Iodized eggs. *Ztschr. f. Ernähr.*, Bd. 2, S. 238.



# A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS <sup>1, 2</sup>

## I. RESULTS OBTAINED IN PRELIMINARY FEEDING TRIALS

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### EIGHT FIGURES

For many years livestock producers in certain localities have complained of unsatisfactory growth and reproduction in domestic animals. As early as 1857, Madison (1860), of the United States Army, noted a severe pathological disturbance among the cavalry horses of his post. The conditions which he described appear to be similar to, if not identical with, the observations which initiated the investigation begun in this laboratory in 1928.

As a result of feeding experience over a number of years, some farmers learned to associate the disturbance with particular tracts of land, although they had various opinions regarding the precise nature of the factors involved. Many believed that the toxic effects were due solely to drinking water, while others attributed the toxic effects to plants growing on their land. Samples of grain from farmers whose livestock had shown pathological symptoms exerted extremely

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<sup>2</sup> These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics of the United States Department of Agriculture.

toxic effects upon white rats. The grain samples were not diseased in any way and macroscopic examination revealed no abnormalities. The grain has no characteristic odor or taste. Routine feed analyses failed to reveal differences between affected and unaffected grains. Only by bioassay could the toxicity of the grain sample be determined.

The effects produced by feeding these toxic grains are apparently not duplicated by any known plant toxins such as the glucosides, toxalbumins, saponins, or alkaloids, nor can the results obtained be attributed to decomposed or contaminated foods. Furthermore, the deaths observed cannot be due to a deficiency of proteins, carbohydrates, fats, minerals, or vitamins. Experimental work indicates that the grains contain a definite toxic compound or compounds. The fact that these toxic effects are produced by grains which have an important place in the diets of both man and animal makes the question of tremendous significance.

In 1931 the results of the experimental work in this laboratory were brought to the attention of the United States Department of Agriculture. As a result of collaborative work, Robinson ('33) reported the occurrence of selenium in wheat and soil samples from affected areas, and Byers ('34) has reported chromium, vanadium, and arsenic in addition to selenium in soil samples from the same region. He also reports the occurrence of selenium and vanadium in a sample of wheat from the same locality. Nelson, Hurd-Karrer, and Robinson ('33) have questioned the use of selenium compounds in insecticides. They found that when selenium was added to soil in even minute quantities, the element was absorbed by wheat plants with the production of a compound extremely toxic to rats and guinea pigs.

A large number of feeding investigations have been conducted in this laboratory during the past several years. Concomitantly, studies of the gross pathology, the physiology, action on enzymes, and chemical nature of the toxic factor are being carried on. As the present paper is of the nature of a preliminary report, the effects produced by toxic grain samples on experimental animals will be stressed.

The experimental animal used in this investigation is the albino rat (*M. norvegicus albinus*) from stock obtained from The Wistar Institute of Anatomy and Biology. It has been found that not only do the rats exhibit wide variations in degree of tolerance, but affected grain samples show all degrees of potency. As the result of many feeding trials, the grain samples have been grouped into two types: lethal and sublethal. The conditions produced in rats by these foodstuffs vary from very severe effects, in which death occurred by the eighth day, to conditions in which growth retardation was the only observed effect. In all the work it has been found necessary to use a rigid control system.

The resistance of the rats to the toxicity was increased by the use of various adjuvants. However, it must be borne in mind that in cases where rats receiving adjuvants showed less pronounced effects, the animals were receiving less of the toxicant per unit of total food consumption. It may be well to state here that the grains from which the following data were obtained were not treated chemically in any way, being merely ground before they were incorporated into various diets.

### *Incidence of death*

By using the thirteen most potent samples of grain (lethal), death was produced in 321 out of 325 rats placed on experiment at various times in the past 4 years. The grains used included corn, wheat, barley, and emmer. Death occurred as follows: 16.3 per cent on or before 25th day of experiment; 38.5 per cent on or before 40th day; 71.1 per cent on or before 60th day; 92.0 per cent on or before 100th day. The largest percentage of the rats (35.7 per cent) died between the 34th and 54th days; 25.2 per cent died between 14th and 34th days, and 16.0 per cent died between the 54th and 74th days of experiment (fig. A).

Six other lots of grain (sublethal) gave very interesting results. Death occurred in only six of fifty-two rats by the sixtieth day. Autopsies on all animals, including those sacri-



ficed, revealed liver lesions which were very striking. Numerous other samples of grain, which do not cause death and may not cause macroscopic lesions, have been encountered. In these samples the only observed effect is depression of growth.

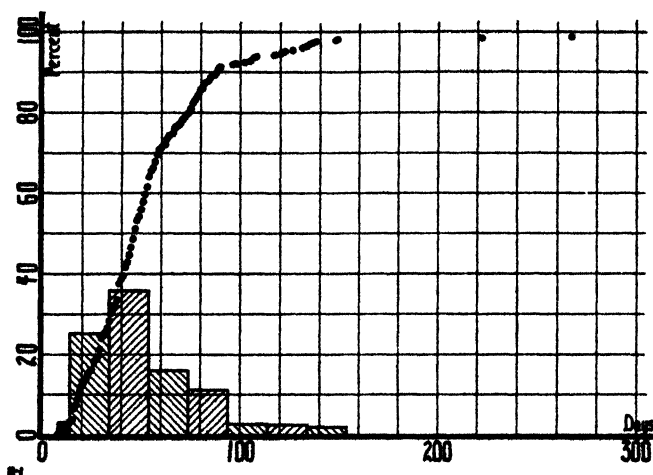


Fig. A Incidence of death in 325 rats on thirteen lethal grain samples. The points show the per cent of the total number of rats which were dead by the corresponding day, and the bars show the percentage of deaths in the indicated 20-day periods. The greatest number of deaths occurred between the thirty-fourth and the fifty-fourth days.

### *Symptoms of pathology*

The symptoms and pathology to be described below are based upon observations and post-mortems on the rats which were used in the feeding trials.

When weaned rats are placed on a diet containing a high percentage of a lethal grain sample they respond immediately by restricting their food consumption to as low as 25 per cent of normal. Some rats lose weight rapidly; others gain weight slowly. Franke and Potter ('34) have shown that the pathology found in the affected animals is not due to inanition, because rats fed identical amounts of control grain show no abnormalities other than stunted growth.

The symptoms appear in a wide variety of forms and it is impossible to describe a definite syndrome which will cover all





cases. Most of the rats soon assume a characteristic hunched posture, and walk in the same position. The fur becomes rough and is usually stained a brilliant yellow around the genitals, due to excessive amounts of bile pigment in the urine. In a few cases the hind legs of the rats become completely paralyzed. In these animals, autopsies usually reveal hemorrhages into the subcutaneous tissues and the muscle fascia near the joints. Figure 1 shows the external appearance at death in a typical case from series 1.

In the first animals that die, the gross pathology is not marked, in comparison with later cases. The most apparent feature is the dilation of the veins in the visceral region. The vena cava and right auricle are almost invariably engorged.



Fig. 1 Appearance of rat no. 58F at death, after 131 days on diet containing lethal corn no. 388 (series 1).

The lungs and the liver usually have a congested appearance. The thymus is greatly degenerated. The reproductive organs are underdeveloped and may show degeneration. The stomach usually has a scanty black residue in it and the small intestines also show evidence of hemorrhage. The animals are usually jaundiced and the bladder is frequently distended with highly colored urine. The animals are always extremely emaciated. The cases just described will be referred to as sub-acute.

The remainder of the rats which die on lethal samples may well be referred to as chronic cases. Although they frequently show moderate increases in weight and may appear

to be in fairly good condition, a definite breakdown of vital organs takes place. These animals develop an anemia of progressive severity and finally die with hemoglobin levels as low as 2.1 gm. per 100 cc. Autopsies reveal striking pathology. The most outstanding feature is in the liver, which is atrophied, necrotic, and hemorrhagic in varying degrees. Figure 2 illustrates a case in which the macroscopic changes in the liver are but slight.

The toxicant evidently produces a definite necrosis of the hepatic cells. Animals with high tolerance are able to sur-

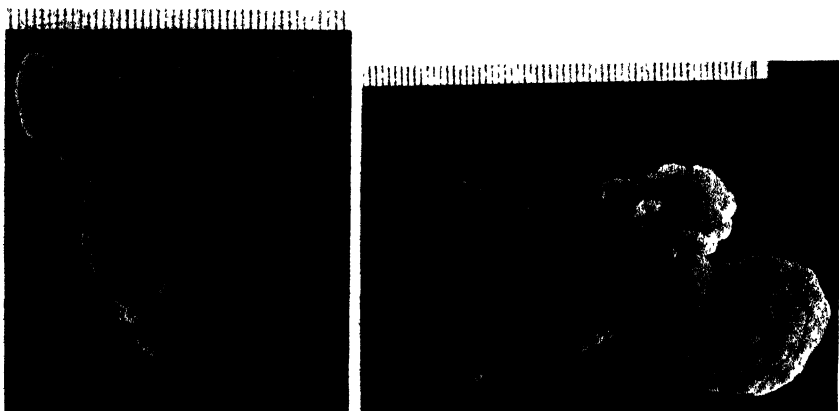


Fig. 2 Liver from rat no. A72F, which was killed after 153 days on a diet containing sublethal corn no. 594 (series 88).

Fig. 3 Liver from rat no. 1885M, which was killed after 365 days on a diet containing lethal wheat no. 582 (series 78).

vive for a longer period of time, because they are able to regenerate their liver cells almost as rapidly as necrosis occurs. It is usually found in regenerative cases that the hypertrophy occurs in either or both the right lateral and the caudate lobes. The left lateral lobe and the central lobes show terrific atrophy, the latter showing the most profound effects. An example of the regenerative type of liver is shown in figure 3, in which the caudate lobes show marked hypertrophy.

The chronic cases also show enlargement of the heart and spleen in many cases. The animals may or may not be jaundiced. Bone conditions resembling vitamin D deficiency

are often found, although the diets have always been adequate in that respect and the controls have developed normally. The bone condition may be analogous to that described by Buchbinder and Kern ('27). The thymus and reproductive organs are affected as in sub-acute cases. The lymph nodes are invariably enlarged and red. The intestinal contents are frequently slate-blue in color. Many of the rats become oedemic. Ascites and pleural oedema are also common. It is felt that the liver is probably one of the primary foci of the toxicant and that the majority of the symptoms are secondary effects. Further evidence for this is found in the fact that in many of the rats which have abundantly regenerative livers, there is very little other pathology. A number of chronic cases have died as a result of an internal hemorrhage from the liver.

The pathology observed in rats on sublethal grain samples is also very interesting. Sub-acute cases are almost never found and the rats which die usually show chronic symptoms.

An interesting case of liver regeneration was shown by a rat on a lethal sample of grain, plus liberal amounts of yeast, orange juice, and cod liver oil (series 20)—(fig. 4).

#### EXPERIMENTAL

It seems desirable to record the details of at least one experiment in this paper. Series 1 was carried out with a lethal sample of corn. Many grain samples show a higher degree of potency than the lot used in this experiment.

An examination of various diets used by different workers showed a great variation in their composition. Since it seemed desirable to have as few outside factors as possible influencing the diet used, the following formula was devised:

	<i>Per cent</i>
Ground whole corn	70.0
Casein	11.0
Sucrose	15.0
Lard	2.0
Calcium carbonate	1.4
Sodium chloride	0.6
<hr/>	
Total	100.0

The casein was prepared by taking a commercial casein and dissolving it in a 0.085 normal sodium hydroxide solution after it had been washed thoroughly with distilled water. The casein was then precipitated with an acid solution of a 1 to 1 mixture of normal hydrochloric and acetic acids. The precipitated casein was washed with distilled water until no test for chlorides could be observed; then it was dried in a



Fig. 4 Liver from rat no. 623F, which was killed after 340 days on a diet containing lethal wheat no. 459 plus 0.4 gm. yeast, 0.15 gm. cod liver oil, and 5 cc. of orange juice daily (series 20).

steam heated drier and ground. The sucrose used was a commercial cane sugar. The lard was Swift's Silver Leaf brand. The salts were chemically pure laboratory reagents. The control and experimental (contained corn lab. no. 388) diets varied only in the source of the corn.

Each rat was kept in an individual cage, as described by Burr and Burr ('29). The rats were weighed every 5 days and the daily food intake recorded.







The rats constituting series 1 were divided into four groups as evenly as possible in regard to age, litter and sex. The four groups in this series were fed as follows:

- Group I Experimental corn no. 388 diet + distilled water.
- Group II Control corn diet + distilled water.
- Group III Experimental corn no. 388 diet + well water (local).
- Group IV Control corn diet + well water (local).

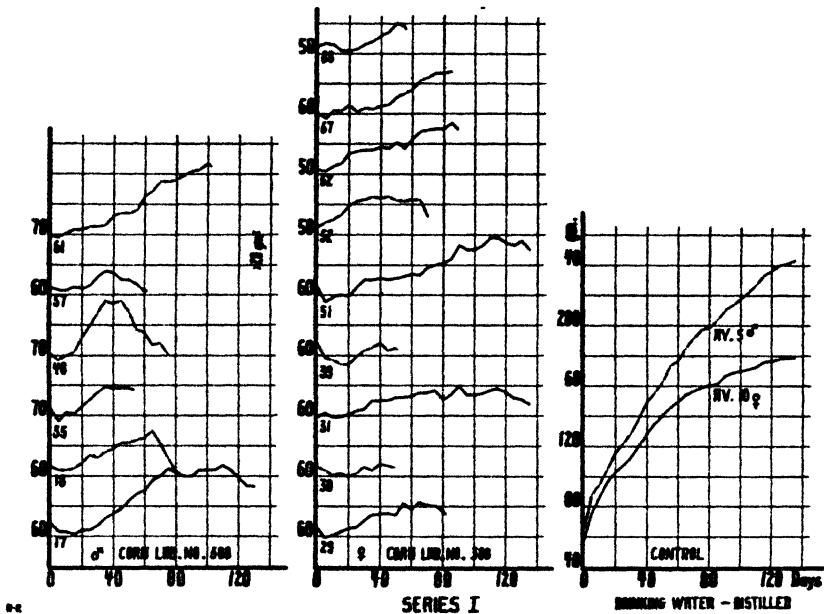


Fig. B Growth curves of rats on toxic corn (lab. no. 388) and average growth curve of rats on control corn. Drinking water, distilled.

The results obtained in series 1 are shown in figures B, C, and D. An examination of the charts indicates clearly that in every case the diet containing corn lab. no. 388 resulted not only in decreased growth, but also in death as well. The control animals showed good growth and were in fine physical condition at the end of the experiment. Figure C, showing the growth curves of the rats receiving well water, seems to show quite distinctly that these rats were more susceptible to the toxicant than were the rats on distilled water (fig. B).

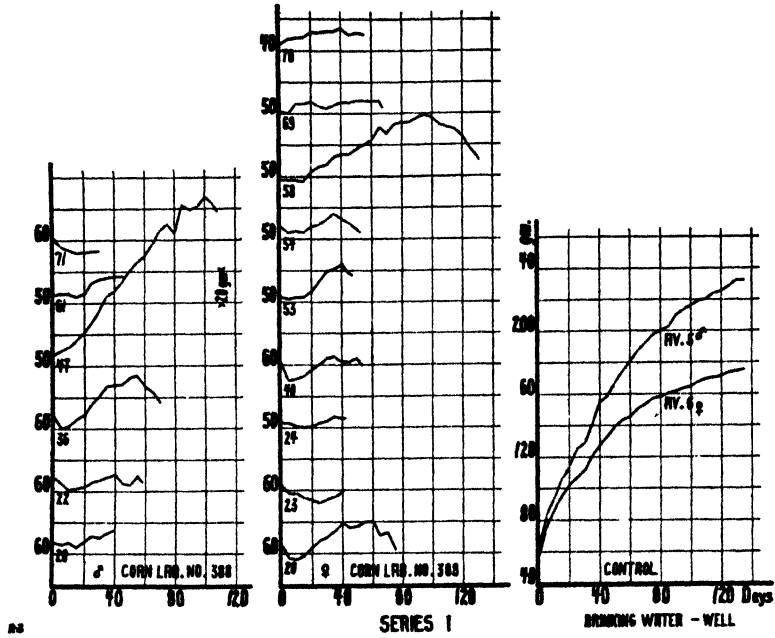


Fig. C Growth curves of rats on toxic corn (lab. no. 388) and average growth curve of rats on control corn. Drinking water, well.

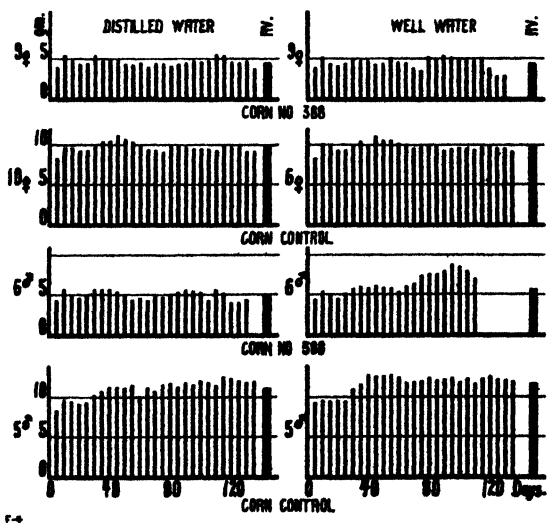


Fig. D The average food consumption of rats in 5-day periods per rat day, and average for entire period (series 1).

The average length of life of males and females receiving toxic food plus distilled water was 85.0 and 84.4 days, respectively, while those receiving toxic food plus well water lived 59.3 and 63.3 days respectively. Autopsies seemed to indicate that the lesions in the rats receiving well water were more severe. Further studies on this point will be made in the future.

As a record of food consumption (shown in fig. D) was kept daily, it was recognized immediately that the rats which obtained the diets containing the corn no. 388 consumed approximately one-half as much as the control groups.

The first rat on the toxic corn succumbed on the thirtieth day and the last on the one hundred and thirty-fifth day. The control rats were all in good condition when killed. The deaths of the experimental rats were not due to starvation, as has been shown by Franke and Potter ('34). Various degrees of pathology, which were described above were noted in the post-mortems of these rats.

#### SUMMARY

1. An apparently new nutritional disturbance, associated with plant material from particular localities, is described.
2. The effects are probably produced by a definite toxic compound or compounds.
3. Thirteen samples of various cereal grains, including corn, wheat, barley, and emmer, from the affected areas produced death in 299 out of 325 white rats within 100 days.
4. Definite pathologic effects produced by the toxic materials are described.

## LITERATURE CITED

- BUCHBINDER, W. C., AND RUTH KERN 1927 Blood calcium deficiency in experimental obstructive jaundice. *Am. J. Physiol.*, vol. 80, p. 273.
- BURR, GEORGE O., AND M. M. BURR 1929 A new deficiency disease produced by the rigid exclusion of fat from the diet. *J. Biol. Chem.*, vol. 82, p. 345.
- BYERS, H. G. 1934 Selenium, vanadium, chromium, and arsenic in one soil. *Ind. and Eng. Chem., News Edition*, vol. 12, p. 122.
- FRANKE, KURT W., AND VAN R. POTTER 1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. III. Hemoglobin levels observed in white rats which are fed toxic wheat. *J. Nutrition* (in press).
- NELSON, E. M., A. M. HURD-KARRER, AND W. O. ROBINSON 1933 Selenium as an insecticide. *Science*, vol. 78, p. 124.
- MADISON, T. C. 1860 Senate Medical Statistics, U. S. Army 1855-1859, War Department, p. 41.
- ROBINSON, W. O. 1933 Determination of selenium in wheat and soils. *J. Assoc. Official Agr. Chem.*, vol. 16, p. 423.

# A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS<sup>1, 2</sup>

## II. THE OCCURRENCE OF THE TOXICANT IN THE PROTEIN FRACTION

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### FOUR FIGURES

After a large number of feeding trials had shown that certain grains produced a nutritional disturbance which could only be caused by some extremely toxic compound or compounds occurring naturally in the plants (Franke, '34) work was begun on the chemical isolation of the toxic factor.

In the summer of 1930, a preliminary study was begun to determine which fraction of the corn (no. 388) carried the toxicant. Corn was extracted with petroleum ether, distilled water, 5 per cent  $K_2SO_4$  solution and 70 per cent ethyl alcohol. After feeding trials using residues from these extractions, it was felt that the protein fraction carried the toxicant. Since it is much easier to separate the protein of wheat, the next experiment was carried out with wheat sample no. 459 which feeding trials had shown to be lethal. This experiment (series 36) gave such conclusive results that it will be described here.

<sup>1</sup> Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 2 from the Department of Experiment Station Chemistry.

<sup>2</sup> These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics of the United States Department of Agriculture.

## EXPERIMENTAL

Whole wheat was ground and the gluten separated from the bran and starch by repeatedly washing in small portions of distilled water. The starch and bran washings were combined and enough sodium hydroxide added to make 0.2 per cent sodium hydroxide solution. The mixture was stirred mechanically for about 4 hours. The suspension was centrifuged in a Sharples supercentrifuge, and the bran and starch were washed twice with distilled water, and dried. The 0.2 per cent sodium hydroxide solution containing the protein dissolved from the starch and bran was used to disperse the gluten. The resulting solution was centrifuged to remove the starch and bran particles. After thorough washing, these were added to the main body of starch and bran previously separated. The protein solution was precipitated at its iso-electric point by adding dilute hydrochloric acid, washed with distilled water twice, and dried.

Both control wheat and wheat no. 459 were treated in this manner and made up into diets (diet no. 3) having the following composition:

	<i>Per cent</i>
Whole ground wheat (separated bran and starch plus precipitated protein)	82.0
Commercial casein	10.0
McCollum's salt mixture no. 185	4.0
Cod liver oil	2.0
Northwestern dehydrated yeast	2.0
Total	100.0

But with the mixtures of protein, starch and bran as follows:

- Group A No. 459 protein plus control starch and bran
- Group B Control protein plus no. 459 starch and bran
- Group C Control protein recombined with control starch and bran

The rats were kept in individual cages described by Franke and Franke ('34). The food intake was recorded daily and the body weight taken every 5 days.

Figures A and B show the individual growth curves of the rats receiving the protein from toxic wheat (group A), as

compared with the composite growth curves of the rats receiving bran and starch from toxic wheat (group B), and the rats receiving both control protein and control bran and starch (Group C). It is quite evident that the bulk of the toxicant is carried in the protein fraction. Experimental indi-

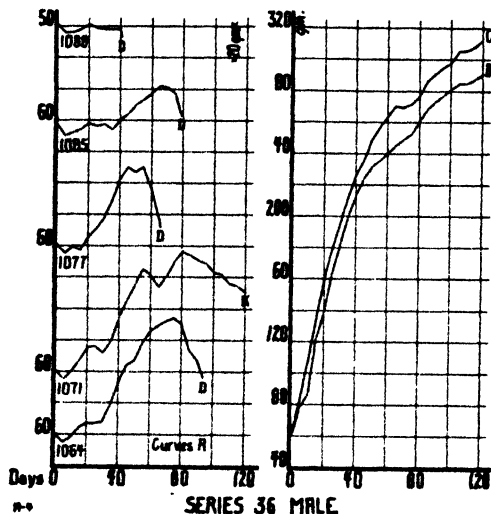


Fig. A Growth of males in series 36: Individual curves—Group A no. 459 protein plus control starch and bran. Average curve—Group B, control protein plus no. 459 starch and bran. Average curve—Group C, control protein recombined with control starch and bran.

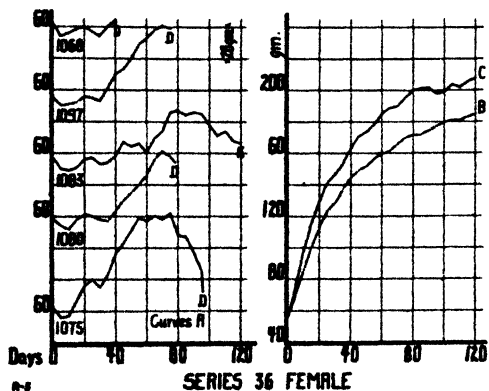


Fig. B Growth of females in series 36: Individual curves—Group A, no. 459 protein plus control starch and bran. Average curve—Group B, control protein plus no. 459 starch and bran. Average curve—Group C, control protein recombined with control starch and bran.



cations are that the growth depression shown in the case of the rats in group B is due to protein which remained in the bran and starch residue. Although the rats in this group showed no pathology, their growth curves are interesting in that they show the growth-depressing effect of small amounts of the toxicant.

It will be noted that the first death occurred on the 41st day, and the last on the 96th day, and that two rats were killed on the 120th day. The postmortems of all the rats showed pathological changes similar to those which have been noted before (Franke, '34). Figures 1 and 2 show the distinctive changes in the livers, spleen, and reproductive organs in typical males and females.

Another experiment was carried out to demonstrate the presence of the toxicant in the protein of corn (series 44). Lethal corn sample no. 523 was extracted with 0.2 per cent sodium hydroxide. The protein was precipitated at its isoelectric point by the addition of dilute hydrochloric acid and combined with the residue from the control corn similarly extracted. The residue from the no. 523 corn was then combined with the control corn protein and the mixture made into diets as in series 36 described above. The results here were not as clear cut as in the wheat experiment, but most of the toxicant was removed by the extraction.

Further studies are being made upon the toxic proteins in an effort to isolate the toxicant. These will be reported in a later paper.

#### SUMMARY

Experiments with samples of toxic wheat and toxic corn have shown that most of the toxicant is carried in the protein fraction of these grains.

#### LITERATURE CITED

- FRANKE, KURT W. 1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. *J. Nutrition*, vol. 8, p. 597.
- FRANKE, KURT W., AND W. ROYDEN FRANKE 1934 A metabolism cage for rats. *J. Lab. and Clin. Med.*, vol. 19, p. 669.

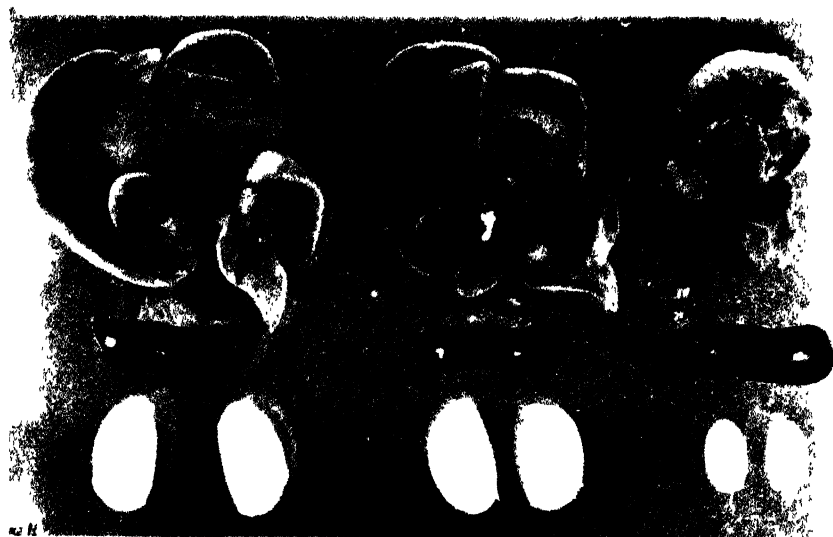


Fig. 1 Relative condition of the liver, spleen and testes from representative litter mates in series 36. Left to right, Group C, B, and A.



Fig. 2 Relative condition of the liver, spleen, uterus and ovaries from representative litter mates in series 36. Left to right, Groups C, B, and A.



# A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS <sup>1,2</sup>

## III. HEMOGLOBIN LEVELS OBSERVED IN WHITE RATS WHICH WERE FED TOXIC WHEAT

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### THREE FIGURES

(Received for publication March 22, 1934)

Franke ('34) has reported the occurrence of an apparently new plant toxicant in various cereal grains as well as native grasses. The toxic factor in the plants is related to the nature of the soil and is endemic to particular localities. The affected plants produce very pronounced effects in livestock and also in experimental animals. Franke showed that of 325 rats placed on lethal samples of grain, 299 had died by the hundredth day. It was observed that many of the rats had an anemic appearance at death.

The present investigation was begun in an attempt to classify the type of anemia encountered and to determine its relation to anemias caused by other toxicants. Preliminary investigations showed that extremely low red cell counts might be expected with correspondingly low red cell volumes and hemoglobin levels. Red cell counts as low as 450,000 cells

<sup>1</sup> Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 7 from the Department of Experiment Station Chemistry.

<sup>2</sup> These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics, of the United States Department of Agriculture.

per cubic millimeter were observed in moribund animals. Blood smears showed that the erythrocytes were very abnormal, with marked anisocytosis and polychromatophilia. The differential leucocyte count revealed marked irregularities in the ratios between the various white cells. Extremely icteric plasmas were found in a large number of affected animals.

Before beginning a complete hematological investigation, it seemed advisable to determine the incidence of the anemia as well as its severity. The hemoglobin estimation was chosen for this purpose, since it gives a very good measure of the degree of anemia present, and requires a minimum of apparatus and time.

Although the toxicant is known to be present in various affected grains, the hematological investigations have been conducted with wheat only.

#### PLAN OF EXPERIMENT

The experimental work was done in two stages. The first step was to determine the hemoglobin levels in moribund rats without previous bleeding (series 85). The majority of the animals were sacrificed when they had become too weak to maintain their equilibrium. Rapid slowing of the respiratory rate was also taken as a criterion of approaching death.

The second step was to determine the rate at which the anemia progressed (series 87). Hemoglobin was determined on tail blood at 3-day intervals.

#### EXPERIMENTAL

The rats used were of Wistar Institute origin. They had been weaned at 21 days and maintained for 1 week on McCollum's Diet I, as described by Burr and Burr ('29). They were then divided into three groups and placed in the individual 'drawer-cages' described by Franke and Franke ('34).

Since the rats receiving the affected wheat diet voluntarily restricted their food consumption to a marked degree, it was

necessary to use a system of double controls, somewhat similar to that described by Swanson and Smith ('32 b). This will be described in detail for series 85.

One group of rats was given the control wheat diet ad lib. These rats served as full controls (group I). Daily food consumption was recorded to the nearest gram. Group II received the diet containing the affected wheat ad lib, and will be referred to as the experimental group. Their daily food consumption was recorded to the nearest tenth of a gram. The rats in group III received the control diet, but their food intake was restricted to the average food consumption of the rats in the experimental group. These rats will be referred to as the restricted controls. It was soon observed that the growth curves of the rats in groups II and III are not strictly comparable due to the wide variations in individual food consumption. Therefore, after the tenth day, each rat in group III received the amount eaten by the corresponding rat in the experimental group. All of the rats were weighed at 5-day intervals until sacrificed. There were ten rats in each group.

Series 87 was organized very much like series 85. The rats were divided into three groups of nine rats each as above. For each experimental rat there was a full control and a restricted control of the same sex, from the same litter, and with approximately the same weight. Food consumption was recorded as in series 85. Each rat in group III received the amount of diet taken by the corresponding experimental rat. Inasmuch as the restricted controls ate their entire quota of diet within 20 minutes after being fed, their hemoglobin values represent virtually 24-hour fasting levels. The rats were weighed and bled at 3-day intervals.

The rats in each of the three groups in series 87 were divided into three sub-groups. Nine rats were bled and weighed every day (three controls, three restricted controls, and three experimental). Since each group contained nine rats, it was possible to bleed each sub-group every third day. By this procedure, individual rats were bled at 3-day intervals, but a continuous daily record of hemoglobin levels for the series could be obtained (fig. 3, which follows).

The high grain diet (diet no. 3) described by Franke ('34) has been modified slightly for the present investigation, and will be given here:

	<i>Per cent</i>
Ground whole wheat	82.0
Commercial casein	10.0
McCollum's salt mixture no. 185	1.0
Pure leaf lard	3.0
Dehydrated yeast (Northwestern)	2.0
Cod liver oil	2.0

The control diet was made with wheat which was considered 'normal,' and the experimental diet was made with wheat (lab. no. 582) classified as lethal (i.e., producing death in the majority of cases by the sixtieth day). This diet gives excellent growth when made with normal wheat.

Fresh distilled water containing a trace of iodine was available to the rats at all times.

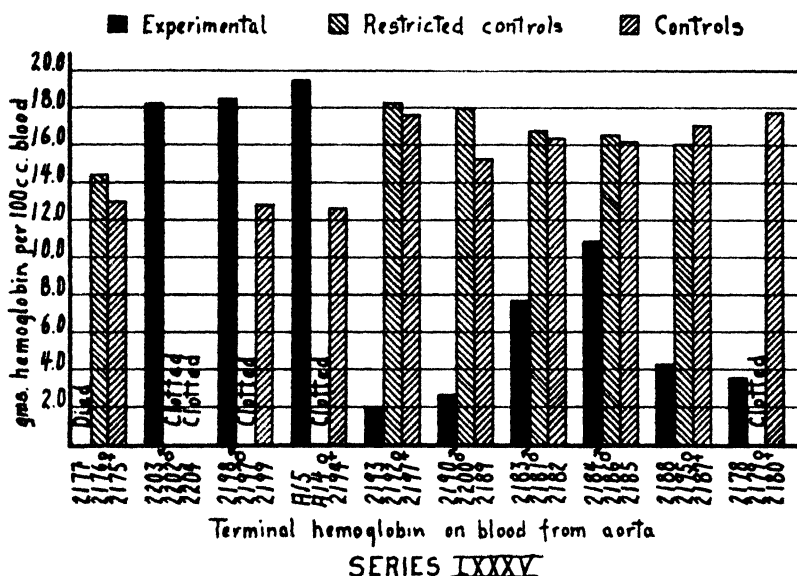
Hemoglobin was determined by the improved Newcomer method, using a Bausch & Lomb standard disc, blue color filter, and colorimeter. Although the authors did not re-standardize the hemoglobin disc, it was felt that the figures obtained would be relatively correct, notwithstanding possible error on the part of the manufacturer. In series 85, the blood was withdrawn from the abdominal aorta by means of a hypodermic needle and syringe, according to Swanson and Smith ('32 a). In series 87, blood was obtained from the rats by clipping off a small portion of the rat's tail. Since only 0.01 cc. of blood is required for the determination, it was possible to make the determinations in triplicate. The same amount of blood was taken from the controls as was taken from the experimental animals. In order to prevent further bleeding, the rat's tails were cauterized with a hot spatula immediately after the samples were taken.

As soon as one of the experimental animals died, both of its controls were sacrificed and the three animals were autopsied. Throughout the experiment, it was possible to compare each experimental animal not only with a normal rat of the same age and sex, but also with a rat which had been maintained on the same plane of nutrition.

## DISCUSSION OF RESULTS

Series 85 showed very plainly that extremely low hemoglobin levels were attained in at least half of the affected rats.

Figure 1 gives the hemoglobin levels which were observed in nine affected rats, when death was about to occur. The figure also gives the hemoglobin levels in the control animals which were sacrificed at the same time. The anemias pro-





remains to be determined. The last six rats (sacrificed on the thirty-fifth day to the seventy-third day of experiment) showed various degrees of anemia with hemoglobin levels ranging from 2.0 to 10.8 gm. of hemoglobin per 100 cc. of blood.

The results obtained in series 87 are shown in figures 2 and 3.

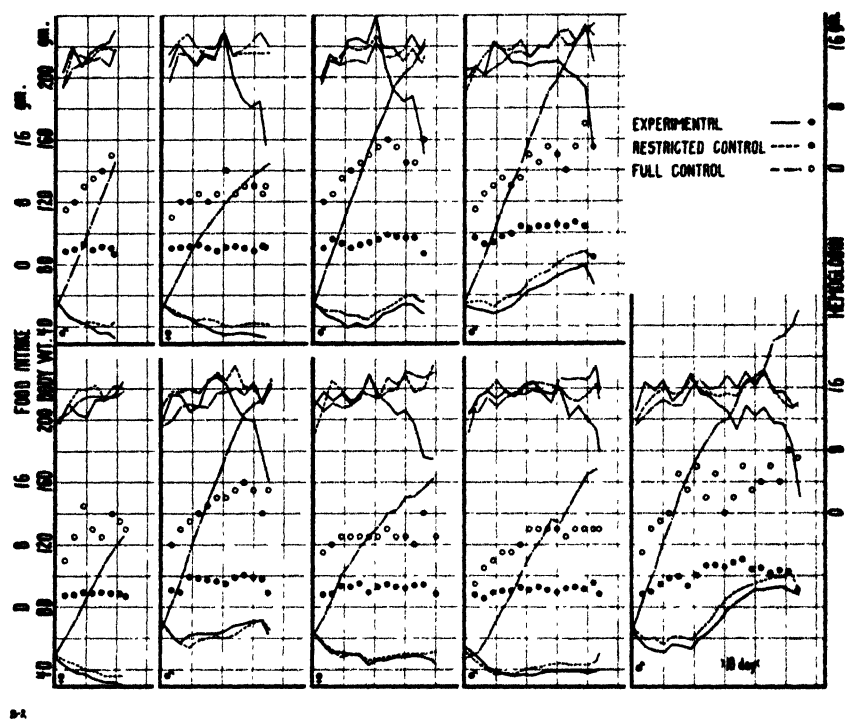


Fig. 2 Hemoglobin, growth and food intake of individual experimental rats and their controls, series 87.

Figure 2 gives the growth, food intake, and hemoglobin levels for each experimental rat together with its two controls for the entire period of the experiment. The upper curves represent hemoglobin levels, and the lower curves represent body weights, while the food intake is represented by unconnected points between the hemoglobin and weight curves in each case. The full controls had the greater food intake in

every instance. It will be noted that by the fifty-fourth day of the experiment all of the experimental rats were dead. The growth curves and food intake records are presented in order to give a clear picture of the plane of nutrition of the experimental animals and the restricted controls in comparison with the full controls. This voluntary inanition on the part of the

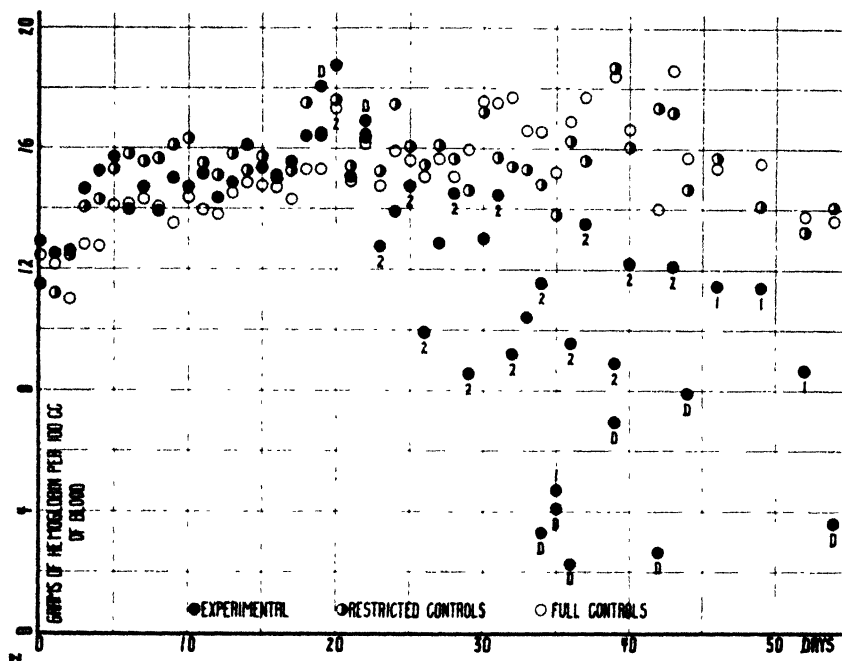


Fig. 3 Daily changes observed in the hemoglobin levels of the 27 rats in series 87. Each point represents the average for one sub-group (three rats except when otherwise indicated by sub-figures). The final hemoglobin levels reached by the nine experimentals are indicated by D.

affected rats will be discussed in another paper. It will be observed that the weights of the experimental rats and the restricted controls approximate each other.

Of the greatest interest is the record of the hemoglobin levels. In spite of the wide fluctuations which are shown in figure 2, the curves show a very definite trend.

Figure 3 presents a composite picture of the hemoglobin levels found in the rats in series 87 for the entire experi-

mental period (54 days). Each point represents the average for three rats (one sub-group as described previously) until deaths begin to occur. The points then represent 1 or 2 rats as indicated by the sub-figures. The chart shows that, beginning with the twenty-third day of the experiment, the experimental animals had hemoglobin levels which were always lower than either of the control groups. Previous to that time, the full control groups showed a definite tendency toward hemoglobin levels which were slightly lower than either the experimental or the restricted control groups. After the experimental rats began to show decreases in hemoglobin, the two groups of controls maintained hemoglobin levels which were not significantly different.

The hemoglobin levels observed in the restricted control seem to indicate that control animals will maintain substantially normal hemoglobin levels when their food intake is restricted to approximately a maintenance level. However, the work here cited involves too small a number of rats to reveal slight differences. Swanson and Smith ('32 b) observed a tendency toward anemia in rats which had their food intake restricted to a lesser degree than the rats in the work here described. Sure, Kik, and Walker ('29) have observed high hemoglobin in cases of inanition, and make the following statement: "This animal shows a typical case of concentration of blood, or anhydremia produced by a progressive marked inanition, as evidenced by the pronounced rise in the concentration of hemoglobin and erythrocytes." Sure, Kik, and Smith ('31) have also noted anemia in this connection, however, stating that "The anemias observed in three animals may be attributable to inanition, since anorexia became quite pronounced in these rats." It must be remembered that in these cases the conclusions were based upon animals which were receiving vitamin deficient diets. It is entirely possible that diverse results may also be due to the degree of inanition which is present.

The decline in hemoglobin, in series 87, occupied a large share of the experimental period. The most rapid fall took

place in 14 days and in the case of the last rat to die (54 days on experiment) the fall lasted for 34 days. It is of interest to note that in this rat, the final drop was due to an internal hemorrhage which caused the hemoglobin to fall from 8.6 to 3.6 gm. per 100 cc. of blood in 36 hours. At autopsy, the abdominal cavity was found to be filled with blood which had come from a lesion in the dorsal caudate lobe of the liver. This was the only case of its kind in either series 85 or 87.

Series 87 substantiates series 85 in that the first rats to die were not anemic. In seven out of nine rats, however, the hemoglobin levels showed a preliminary rise which was followed by a gradual decline terminated by a sudden drop to fatal levels. The pathology observed in the experimental animals was typical and has been described by Franke ('34).

There may be some objection to bleeding the rats at 3-day intervals. The fact that control animals showed no decline in hemoglobin, together with the fact that the low hemoglobin levels in series 85 were achieved without previous bleedings, would indicate that the anemias encountered are due solely to some factor in the affected wheat.

Although the blood plasma was not icteric in all cases, there was pronounced icterus in the two rats which died with high hemoglobin values. This indicated that either extensive blood destruction had begun or marked liver damage had taken place. Of the other seven rats, only two had plasmas which were not icteric at death. In these two cases, the anemia was probably due to lack of blood formation.

The high icteric indices and the jaundice found in affected rats do not necessarily imply increased blood destruction. It must be borne in mind that extensive liver damage is taking place, and that the high icteric indices, the jaundice, and the urobilinuria may be due to this factor. Jordan and Greene ('30) noted the anemias associated with both clinical and experimental jaundices and expressed the belief that the anemia was due to lack of blood formation. They stated that, although bile salts are hemolytic, their concentration in the blood stream is never sufficient to cause hemolysis. If this be

true, the anemias reported above must be due to lack of blood formation, to direct hemolytic action on the part of the toxicant, to hyperactivity of the normal blood cells destroying mechanisms, or to a combination of these factors. Experiments are being planned which will attempt to show which of the above effects is responsible for the anemia encountered.

#### SUMMARY

1. Death occurred in all of nineteen rats placed on an otherwise adequate diet containing toxic wheat.

2. The first six deaths occurred with no decline in hemoglobin and the last thirteen deaths occurred with hemoglobin levels ranging from 2.0 to 10.8 gm. per 100 cc. of blood.

3. The falls in hemoglobin extended over periods ranging from 14 to 34 days.

4. The anemia observed was not due to inanition.

#### LITERATURE CITED

- BURR, G. O., AND M. M. BURR 1929 A new deficiency disease produced by the rigid exclusion of fat from the diet. *J. Biol. Chem.*, vol. 82, p. 345.
- FRANKE, KURT W. 1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. *J. Nutrition*, vol. 8, p. 597.
- FRANKE, KURT W., AND W. R. FRANKE 1934 A metabolism cage for rats. *J. Lab. and Clin. Med.*, vol. 19, p. 669.
- JORDAN, F. M., AND C. H. GREENE 1930 Anemia in jaundice. II. The formation of hemoglobin in experimental obstructive jaundice. *Am. J. Physiol.*, vol. 91, p. 409.
- SWANSON, P. P., AND A. H. SMITH 1932 a Total nitrogen of the blood plasma of normal albino rats at different ages. *J. Biol. Chem.*, vol. 97, p. 745.
- 1932 b Inorganic salts in nutrition. IV. Changes induced in the blood by a ration deficient in inorganic constituents. *J. Biol. Chem.*, vol. 98, p. 479.
- SURE, B., M. C. KIK, AND D. J. WALKER 1929 The effect of avitaminosis on hematopoietic function. I. Vitamin A deficiency. *J. Biol. Chem.*, vol. 83, p. 375.
- SURE, B., M. C. KIK AND M. E. SMITH 1931 Hematopoietic function in avitaminosis. VI. Vitamin G deficiency. *Proc. Soc. Exptl. Biol. and Med.*, vol. 28, p. 498.

# A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS<sup>1,2</sup>

## IV. EFFECT OF PROTEINS ON YEAST FERMENTATION

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### THREE FIGURES

(Received for publication March 22, 1934)

These studies on enzyme activity were started with two primary objects in mind. The first was to develop a simple biological test for certain protein toxicity described by Franke ('34), and the second to study the possible effects of these toxic proteins on several of the important enzymes of the body.

At the time these investigations were started, the only positive test available for the testing of foodstuffs for this toxicant was that of feeding trials on animals. This test gives satisfactory results, but has the disadvantage of being expensive and time consuming, besides requiring large amounts of material for a single test. To be of greatest use it is very desirable that a biological test for this toxicant possess the following qualities: 1. Economy of material; 2. rapidity of completion; 3. quantitative measurement of toxicity.

<sup>1</sup> Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 6 from the Department of Experiment Station Chemistry.

<sup>2</sup> These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics, of the United States Department of Agriculture.

The question of the effects of the toxicant upon enzymes arose in connection with the feeding trials with rats. Early in this phase of the work, it was recognized that experimental animals ate only half as much as control animals (Franke, '34).

This voluntary food restriction certainly cannot be attributed to factors, such as monotony of diet, physical texture, lack of roughage, etc., since it took place within 24 hours after the rats were given the diet. The palatability of the diet may be questioned, but human taste and smell are incapable of differentiating between control and affected grains. There remains the possibility that the affected diet does not stimulate the appetite or digestion either by not increasing the flow of enzymes or else by rendering them inactive.

In order to get some information as to the possible effects of the toxicant on the enzymes in the animal body, it was decided to investigate the effect of the toxicant upon various enzymes *in vitro*.

The rate of fermentation of glucose by yeast in the presence of the material to be tested was the first enzymatic reaction to be tried, since the rate of this reaction is probably much more simple to measure than the rates of most other enzymatic reactions. It is interesting to note that Branham ('29 a, b), as well as earlier workers, made use of this enzymatic reaction to compare the antiseptic value of various compounds.

Several preliminary trials were carried out in ordinary fermentation tubes. These tubes contained sugar solution, yeast, and 1 gm. of wheat gluten. They were allowed to stand at room temperature, and at the end of 5 hours, measurements were made of the volume of CO<sub>2</sub> produced. The tubes containing gluten from control wheat produced 58 per cent more CO<sub>2</sub> than those tubes containing gluten from wheat which feeding trials had shown to be toxic.

Zeller ('26) found that solutions containing proteins caused increased rates of fermentation. He found that amino acids varied in their effects from depressing to a 100 per cent increase in rate of fermentation. Neuberg and Kobel ('26) stated that a mixture containing an amino acid and a sugar

is, as a rule, more quickly fermented by yeast than a solution containing sugar alone. They also stated that the fermentation of sugar by yeast juice is inhibited by the amino acids and urea.

Schroeder, Woodward, and Platt ('33) found that yeast poisoned by sodium iodoacetate was reactivated by addition of certain amines and amino acids, but that this reactivation seemed to be due to the change of pH when these were added, rather than a specific action of amines and amino acids.

#### EXPERIMENTAL

*Apparatus.* Although fermentation tubes, or similar apparatus, have been used by other workers for studies on the rate of fermentation, the preliminary trials on this problem were so promising that it was thought justifiable to construct the apparatus described by Franke and Moxon ('34).

The fermentation was carried out in a thermostat at a temperature of  $28^{\circ}\text{C.} \pm 0.05^{\circ}\text{C.}$  Other workers have used higher temperatures for studies on the rate of fermentation. Richards ('28) found that at temperatures above  $30^{\circ}\text{C.}$  a decrease in the rate of growth of yeast cells is associated with an increase in temperature, and also that, at  $30^{\circ}\text{C.}$  abnormal, elongate cells appear which indicates that this temperature affects the bud forming process in a critical manner. On the other hand, one must not forget that division and growth of yeast cells and fermentation are altogether different processes, and that  $30^{\circ}\text{C.}$  or higher temperatures, within certain limits, may be entirely satisfactory for fermentation studies. Stier ('33) states that "Temperatures below  $30^{\circ}$  would be nearer the range of 'normal' physiological activity for yeast and similar organisms." Waksman and Davidson ('26) give the optimal temperature for zymase activity to be between  $28^{\circ}$  and  $30^{\circ}\text{C.}$

*Materials.* Distilled water was used in every case where water was required. Glucose—Merck's C.P. anhydrous. Yeast—small cakes of Fleischmann's yeast were purchased from a local store twice a week. These were stored in a re-



frigerator at about 0°C. until used. Even under these precautions, variations in fermenting power of individual cakes from day to day were encountered. Therefore, controls were run with every experiment.

*Proteins.* Corn protein was extracted from the ground grain with 0.2 per cent NaOH according to the following method: 1000 gm. of ground grain was stirred into a battery jar containing 7 liters of 0.2 per cent NaOH and allowed to stand, with occasional stirring, for 5 hours. The supernatant liquid was then siphoned off and run through a Sharples super-centrifuge to remove all suspended particles. The protein was precipitated from the NaOH solution by adding dilute HCl until the isoelectric point was reached. The flocculated protein was allowed to settle overnight and the supernatant liquid was then siphoned off and discarded. The excess solution was removed from the precipitate by centrifuging and the protein precipitate was then dried at a low temperature. The protein was ground before being used.

*Wheat gluten* was prepared by soaking ground wheat in a small amount of distilled water for  $\frac{1}{2}$  hour and then washing the bran and starch from the gluten by kneading in several portions of distilled water. The gluten, before it was used, was dried at a low temperature and ground.

*Amounts of material used.* At the beginning 1 gm. of sugar was arbitrarily chosen as a convenient amount to use in this study. Likewise, 1 gm. of protein was used. In the case of yeast it was thought desirable to make a few preliminary trials before deciding on the quantity to use, as follows:

A. Control—1 gm. glucose, 1 gm. control corn protein, 20 cc. of 10 per cent yeast suspension.

B. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 20 cc. of 10 per cent yeast suspension.

C. Control—1 gm. glucose, 1 gm. control corn protein, 10 cc. of 10 per cent yeast suspension, 10 cc. distilled water.

D. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 10 cc. of 10 per cent yeast suspension, 10 cc. distilled water.

E. Control—1 gm. glucose, 1 gm. control corn protein, 5 cc. of 10 per cent yeast suspension, 15 cc. distilled water.

F. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 5 cc. 10 per cent yeast suspension, 15 cc. distilled water.

G. Control—1 gm. glucose, 1 gm. control corn protein, 2.5 cc. 10 per cent yeast suspension, 17.5 cc. of distilled water.

H. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 2.5 cc. 10 per cent yeast suspension, 17.5 cc. of distilled water.

The yeast used was from the same cake.

The results of the above fermentation are plotted in figure 1.

The curves indicate that 2.5 cc. of 10 per cent yeast suspension gives the widest range between control and experi-

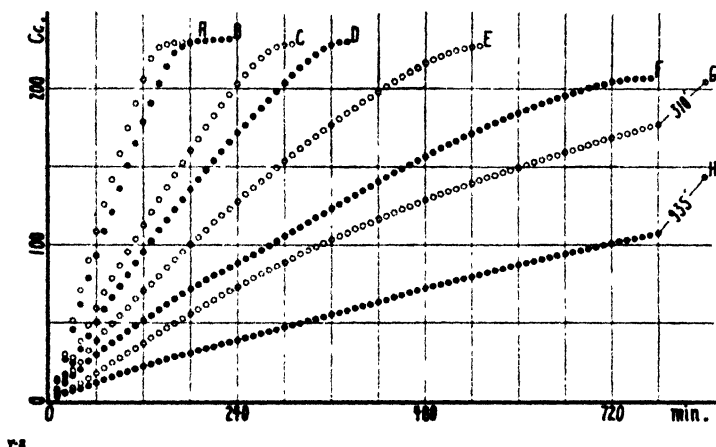


Fig. 1 The rate of  $\text{CO}_2$  production as influenced by different corn proteins and different amounts of yeast.

mental rates of fermentation. However, the 5 cc. of yeast gives a fairly wide range and ferments much faster so that complete results can be obtained in a shorter time. Therefore, 5 cc. of 10 per cent yeast suspension was used in subsequent experiments.

Figure 2 shows the rate of  $\text{CO}_2$  production by—

A. 1 gm. glucose, 1 gm. control corn protein, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

B. 1 gm. glucose, 1 gm. no. 523 corn protein, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

C. 1 gm. glucose, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

The curves indicate that the stimulating effect on fermentation is missing from the affected corn protein, except for the

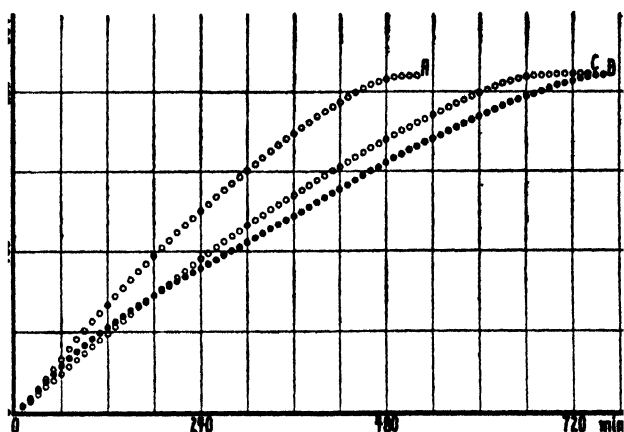


Fig. 2 The rate of  $\text{CO}_2$  production by, A. Control corn protein. B. No. 523 corn protein. C. No protein.

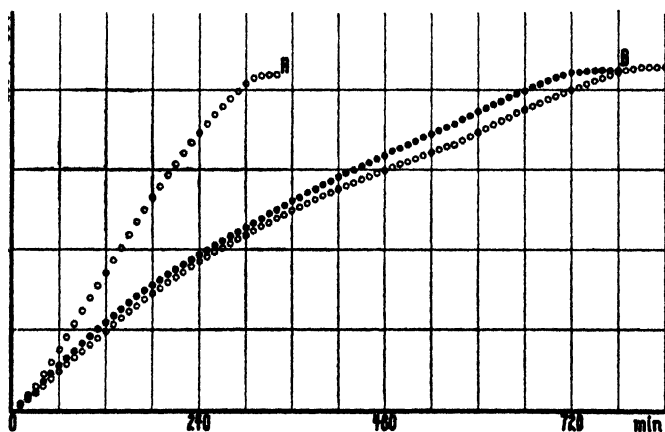


Fig. 3 The rate of  $\text{CO}_2$  production as influenced by, A. Control wheat gluten. B. No. 582 wheat gluten. C. No protein.

first  $2\frac{1}{2}$  hours. This may indicate the slow inactivating of some of the yeast cells either by retardation of activity or even by death.

Figure 3 shows the rate of  $\text{CO}_2$  production by—

A. 1 gm. glucose, 1 gm. control wheat gluten, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

B. 1 gm. glucose, 1 gm. no. 582 wheat gluten, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

C. 1 gm. glucose, 5 cc. 10 per cent yeast suspension, and 15 cc. distilled water.

These curves show again that the protein from the affected foodstuffs does not have the stimulating effect shown by the control.

When comparison is made between control wheat and control corn protein, the wheat has the greater stimulating effect. However, one must remember that the composition of these varies.

It was early recognized, as has been observed by others, that the hydrogen ion concentration, temperature and composition of buffers when used would influence the rate of fermentation. Papers are in preparation covering these factors as well as the effect of toxic protein on other enzymes—pepsin, trypsin, invertase, etc.

#### SUMMARY

1. Protein from a 'normal' grain when added to a fermenting mixture of yeast and glucose will increase the rate of reaction.

2. Protein from an 'affected' grain will not increase the rate of fermentation.

3. Proteins from wheat appear to give a greater stimulating effect than proteins from corn.

4. This accelerating effect of protein varies with the protein-yeast ratio.

## LITERATURE CITED

- BRANHAM, SARA E. 1929 a An improved technique for the comparison of anti-septics by yeast fermentation. *J. Infect. Dis.*, vol. 44, p. 142.
- 1929 b The effects of certain chemical compounds upon the course of gas production by baker's yeast. *J. Bact.*, vol. 18, p. 247.
- FRANKE, KURT W. 1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. *J. Nutrition*, vol. 8, p. 597.
- FRANKE, KURT W., AND ALVIN L. MOXON 1934 An apparatus for determination of the rate of CO<sub>2</sub> production during yeast fermentation. *J. Biol. Chem.*, vol. 105, p. 415.
- NEUBERG, C., AND M. KOBEL 1926 Weiteres über die Reaktion einzelner Zuckerarten mit verschiedenen Aminosäuren nebst Bemerkungen über die Vergärung von Aminosäuren-Zucker-Gemischen. *Biochem. Z.*, Bd. 174, S. 464. Cited in *Biol. Abstracts*, vol. 1, abst. no. 11501.
- RICHARDS, OSCAR W. 1928 The rate of the multiplication of yeast at different temperatures. *J. Physiol. Chem.*, vol. 32, p. 1865.
- SCHROEDER, E. F., GLADYS E. WOODWARD, AND MURIEL E. PLATT 1933 The effect of amines on yeast poisoned by iodo-acetic acid. *J. Biol. Chem.*, vol. 100, p. 525.
- STIER, T. J. B. 1933 The rate of oxygen utilization by yeast as related to temperature. *J. Gen. Physiol.*, vol. 16, p. 815.
- WAKSMAN, S. A., AND W. C. DAVIDSON 1926 *Enzymes*. The William & Wilkins Company, Baltimore.
- ZELLER, HEINRICH 1926 Wirkung N-haltiger Substanzen auf die Hefegärung. V. *Biochem. Ztschr.*, Bd. 176, S. 134. Cited in *Biol. Abstracts* vol.1, abst. no. 12613.

# THE EFFECT OF PRUNES AND THE WATER EXTRACT OF PRUNES ON THE PLASMA CO<sub>2</sub> COMBINING CAPACITY AND COMPOSITION OF THE URINE WHEN INCLUDED IN ACID, NEUTRAL AND UNCONTROLLED DIETS

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## INTRODUCTION

The early experiments of Sherman and Sinclair ('07) and Sherman and Gettler ('12), based on analytical data, found prunes to have an excess of basic elements equal to 24.4 cc. of normal acid per 100 gm. of prunes. However, feeding experiments of Blatherwick and Long ('14 and '23) showed that prunes, though yielding a basic ash, nevertheless increased the acid formation. The decrease in urinary pH caused by eating prunes was attributed to hippuric acid resulting from benzoic acid or a precursor of benzoic acid, such as quinic, present in prunes. Brunton and Wilson ('32) stated that, contrary to expectations, both raisins and prunes, which have a high excess of alkali, produce no alkaline tide, but may produce an acid tide.

Aside from the forementioned literature, no information concerning the effects of prunes on the plasma CO<sub>2</sub> combining power or composition of the urine was found. In view of this absence of information correlating effects of prunes on blood plasma and composition of the urine, and in the light of the more recent findings of Fasold ('30, '31 a, '31 b, '31 c), Eimer ('31) and Bischoff et al. ('32, '34), Michalowsky ('30),

Samuel and Kugelmass ('30), and Schwartz and Dibold ('31), several experiments were conducted to determine the effects of prunes and the water extract of prunes on the plasma  $\text{CO}_2$  combining capacity and the composition of the urine.

#### EXPERIMENTAL

The effect of prunes and the water extract of prunes (prune juice) on the plasma  $\text{CO}_2$  combining power and composition of the urine was determined for a neutral, acid and uncontrolled diet. The acidities of the diets were calculated from the table of Sherman and Gettler ('12). This is not an exact method, as pointed out by Salter, Fulton and Angier ('31), and an error of 10 cc. or more of N. acid may quite commonly arise in calculating the potential acidity or alkalinity of a diet. It is also pointed out that cooking may change the acidity of a food. Eimer ('31) also indicated the danger of judging diets to be acid or basic on the basis of chemical ash analyses alone. Nevertheless, the diets used in our experiments were comparative, even though the calculations may have been in error several per cent.

An attempt was made to have a reasonably balanced diet especially in respect to edibility and nutritive value. All food was prepared for eating by the writers, except the bread.

Eight healthy young men continued on the neutral control diet for 4 days and then 6, 12, or 18 prunes or prune juice were included in the diet each day as designated in table 1. The prunes used were of the French variety from Santa Clara County of 40-50 size canned by the writers in syrup in order to obtain a uniform product. The ash of the prunes was equal to 66.32 cc. of N. base per 100 gm. of prunes. The juice used in the tests was made by extracting dried prunes with water in accordance with the procedure of Mrak and Cruess ('29). One hundred and twenty cc. of juice was equivalent to about 50 gm. of prunes and the ash was equal to 38 cc. of N. base per 100 cc. juice. An attempt was made to control the amount of water ingested and as far as possible the physical exertion of each individual. However, there

undoubtedly were variations in physical exertion that caused variations in the results obtained. We are aware of some of them. It has been pointed out by Barr ('22), Dennig ('31), Rice and Steinhaus ('31), and Bischoff et al. ('34) that exercise is a very important factor in causing variations in the alkaline reserve of the blood.

TABLE 1

*Condensed table, showing the averages during the control and prune period, respectively*

SUBJECTS	NUMBER OF PRUNES IN DIET	C VOLUME	C + P VOLUME	C 0.1-N TITRATABLE ACIDITY	C+P 0.1-N TITRATABLE ACIDITY	C 0.1-N ORGANIC ACID	C+P 0.1-N ORGANIC ACID	C NH <sub>3</sub> -N	C+P NH <sub>3</sub> -N	C pH	C+P pH	C CO <sub>2</sub> COMBINING CAPACITY	C+P CO <sub>2</sub> COMBINING CAPACITY
Acid diet													
1.-E.M.	6	1250	1574	155	242	654	785	130	142	6.11	5.81	67.44	67.08
2.-C.S.	Juice	970	1100	265	275	636	692	163	194	5.95	5.72	66.24	65.19
3.-J.F.	6	969	1358		183	606	723	203	246	5.96	5.90	63.80	64.60
4.-B.C.	12	1093	1154	238	222	556	664	133	125	5.88	5.93	59.96	61.72
5.-R.D.	18	923	1176	292	299	495	738	132	142	5.60	5.50	64.56	65.32
6.-J.D.	18	1375	1708	112	176	593	898	153	220	6.08	5.78	61.17	61.84
7.-W.C.	12	1093	1154	238	222	556	664	133	125	5.88	5.93	59.96	61.72
8.-C.H.	Juice	1370	1210	174	212	607	648	166	138	5.93	5.72	66.00	64.82
Neutral diet													
1.-E.M.	6	1369	1405	127	126	665	662	204	109	6.08	6.05	68.09	68.85
2.-C.S.	12	795	900	124	167	544	644	208	185	6.16	5.97	68.09	66.65
3.-J.F.	18	915	1246	151	124	507	714	102	143	6.04	5.99	64.96	65.66
4.-B.C.	Juice	1210	1090	169	135	630	590	149	59	5.95	5.82	61.78	62.73
5.-R.D.	18	1065	1000	156	218	521	545	100	104	5.98	5.74	63.23	63.88
6.-J.D.	12	1197	1470	108	128	541	617	172	177	6.08	5.91	67.61	66.47
7.-A.L.	6	1500	1457	107	102	660	620	176	144	6.14	5.77	63.10	68.07
8.-C.H.	Juice	829	1140	126	142	540	590	206	95	5.70	5.90	64.40	66.60

O = on control diet.

C + P = on control diet plus prunes.

Urine samples were collected for the 24-hour period starting after breakfast and ending before breakfast. Blood samples were drawn between 5.00 and 8.00 P.M. except for subjects 5, 6 and 7 on the last day. Blood samples were taken from three individuals on the last day of the neutral diet to determine the changes occurring in plasma CO<sub>2</sub> combining capacity at various intervals after the ingestion of prunes.



The procedure used for the experiment on the acid diet was similar to that used for the neutral diet except that extra blood samples were not taken the last day. A glass of water was included between meals and after dinner.

The calculated excess acidity for the diet was more than 50 cc. N. acid. This is a less acid diet than employed by Bischoff et al. ('34), but more acid than used by Fellers et al. ('33) which we calculated to be equal to 11.2 cc. N. acid. The diet used by Chaney and Blunt ('25), Saywell ('32 a, b and '33), and Saywell and Lane ('33) is equal to about 20.42 cc. of N. acid according to our calculations from the tables of Sherman and Gettler.

In the uncontrolled diet no attempt was made to control the activities of the men or the food they ate. A record was kept of the food eaten at each meal and a rough estimation was made of the acid or base of the meal based on Sherman and Gettler's tables. The averages of these results are included in table 2 under the designations of A for acidic, B for basic and N for neutral. The letters A or B alone indicate

TABLE 2

*Condensed table, showing the averages for the uncontrolled diet*

SUBJECTS	NUMBER OF PRUNES IN DIET	ACID BASE OF DIET		C VOLUME	C+P VOLUME	C 0.1-N TITRATABLE ACIDITY	C+P 0.1-N TITRATABLE ACIDITY	C 0.1-N ORGANIC ACID	C+P 0.1-N ORGANIC ACID	C NH <sub>3</sub> -N	C+P NH <sub>3</sub> -N	C pH	C+P pH	C CO <sub>2</sub> COMBINING CAPACITY	C+P CO <sub>2</sub> COMBINING CAPACITY
		C	C+P												
1	18	A ±	A ±	1003	935	13	21	640	534	20	37	6.48	6.77	66.66	66.00
2	Control	N	N	1217	1278	84	127	572	492	43	68	6.13	6.37	64.42	63.60
3	12	A +	A +	1854	2020	110	176	528	550	29	52	5.75	6.14	65.45	65.74
4	12	A +	A	587	492	107	135	395	347	11	41	5.89	5.84	61.50	59.76
5	18	A ±	A +	1420	1517	217	229	575	589	45	113	5.72	5.69	64.10	62.66
6	6	A +	A +	2148	2087	171	133	808	754	66	123	6.09	6.08	63.70	59.73
7	Juice	A +	A +	1970	2100	162	208	626	735	123	79	6.16	6.11	62.92	62.76
8	Juice	A ±	A +	993	1245	207	79	431	569	40	53	5.56	6.20	64.45	64.54
9	Juice	A -	A -	1153	1515	111	184	734	663	24	59	6.25	6.07	67.17	63.72
10	6	A	A ±	932	972	66	-14	409	558	24	56	6.13	6.69	62.49	59.19
11	Control	A ±	A +	1013	1034	140	102	479	520	43	57	6.26	6.51	67.93	66.12
12	Control	A +	A ±	1647	1995	141	160	760	777	57	191	6.17	6.34	68.49	64.10

Subject 8 fasted 3 days during the control period.

Subject 1 found to be a NaHCO<sub>3</sub> addict.

Subject 10 took NaHCO<sub>3</sub> on two days.

a very mildly acid or basic meal, whereas the presence of one + indicates acidic or basic food equal to about 10–25 cc. of N. acid or base. Two + marks (i.e., A++) indicates an acid or basic meal stronger than 25 cc. of N. acid or base. N— indicates an acid to neutral meal and N+ indicates a neutral to basic meal. Most of the meals ingested during the period of this diet were acid according to our calculations. Twelve men ranging in ages from 18 to 72, engaged in working in a prison hospital, were included in the diet for a period of 15 days. The first 7 days was a prune free period to obtain an idea of the normal variation in the plasma CO<sub>2</sub> binding power and composition of the urine. During the second period of 8 days various amounts of prunes or 120 cc. of prune juice was included in the diet of nine of the subjects. Three of the men continued through the second period without including prunes or prune juice in their diet. Urine samples were collected for the 24-hour day starting after breakfast and ending before breakfast the next morning. Blood samples were taken at 9.00 A.M. each morning.

Analytical determinations were all made shortly after the collection of the samples. All determinations were made in duplicate or triplicate, except those of the ammonia, which in most cases were single determinations. The following methods were employed: pH by the quinhydrone electrode; organic acids, Van Slyke and Palmer ('20); ammonia, Van Slyke and Cullen ('14); titratable acidity, Henderson ('11); and plasma CO<sub>2</sub>, Van Slyke and Cullen ('17), as described by Hawk and Bergeim ('31), page 489.

#### RESULTS AND DISCUSSION

*Neutral diet.* The results obtained for the neutral diet are given in a summary of the averages in table 1.

There was considerable variation in volume excreted even though an attempt was made to control it by regulating the daily intake of water and the activities of the men. These variations undoubtedly had some effect on the pH values of the urine by changing the buffer relations; the reasons for

this, however, are beyond the scope of these investigations. The average pH values for the control periods had an extreme variation of 0.46 pH units which is 0.05 less than the extreme variation for any individual during that period. In most instances the extreme individual variations for the control period were not over 0.27 pH units. The pH values for the control periods all fall within a range of 5.7 to 6.16. This appears to be considerable variation; however, the urinary pH values of the individual subjects varied, some being consistently lower and others higher. The writers believe a fairly stable state was reached when prunes were included in the diet. In all instances when prunes were included in the diet there was a drop in the pH of the urines. In no instance, however, did the average pH of the urine of an individual for the control and prune (C + P) period, fall below the lowest average pH obtained for an individual (subject 8) during the control period. The changes obtained when juice was included in the neutral diet were not consistent. Subject 8 showed an average increase of 0.2 pH units and subject 4 an average decrease of 0.13 pH units. These changes are probably not significant. There was no direct correlation between the pH of the urine and the amount of prunes ingested on the neutral diet. It is possible that if more men had been used for each particular test the variations would have been more consistent; however, since the variations were so wide we do not believe it would have made any difference.

The titratable acidity of the urine did not change consistently. Variations were observed, but are believed to have been within the normal ranges. More significant is the organic acid output. A marked change in organic acid excreted was observed when twelve and eighteen prunes were included in the diet in all instances except in the case of subject 5. Only slight changes in organic acid excreted occurred when six prunes or 120 cc. of prune juice were included in the diet. It should be pointed out here that, according to the recent work of Fasold ('30), there is a relation between the organic

acid excreted and the kind of food eaten. He indicated that organic acids, as citric and lactic, are mostly oxidized and that a relation exists between the amount of basic constituents of foods ingested and organic acid excreted. The increased organic acid excretion observed when twelve and eighteen prunes were included in the neutral diet was not caused by the presence of benzoic acid, according to findings of Blatherwick and Long ('23). They believed the increase in organic acid excretion occurring upon the ingestion of prunes to be caused largely by quinic acid in the prunes. Fellers et al. ('33) attributed the increase in organic acid excretion caused by cranberries largely to quinic acid in the fruit. Quick ('31) found quinic acid to be a precursor of benzoic acid which is excreted as hippuric acid. In the absence of more definite information regarding the quinic acid of prunes, it appears more reasonable to interpret the increase in organic acid excretion caused by eating prunes in the light of the findings of Fasold ('30, '31 a '31 b, '31 c). According to his interpretations, the increased excretion of organic acids observed when twelve and eighteen prunes were ingested was caused by the excess alkalinity of the prune plus neutral diet even though some hippuric acid forming substances as benzoic acid were present in the prunes. As previously stated, prune ash has a relatively high alkaline value, whereas the amount of benzoic acid, as given by Radin ('14), and the small amount of quinic acid probably present hardly seem sufficient to account for the marked increase in hippuric acid excretion observed. Further studies on hippuric acid and urinary pH changes are being made and will be reported at a later date.

In regard to the NH<sub>3</sub> eliminated during the neutral diet, the most marked decrease was observed in the case of those ingesting prune juice. However, the subjects ingesting prunes showed a slight decrease, if any, in the free ammonia. In the light of Fasold's findings, there must have been sufficient alkali in the prunes and the prune juice to replace the difference in the NH<sub>3</sub> excreted when the NH<sub>3</sub> excretion decreased after including prunes in the diet. Fasold and others have

shown that  $\text{NH}_3$  is called upon for the neutralization of acids to some extent and acids are called upon for the neutralization of bases to some extent. This agrees with the forementioned interpretation of the observations on organic acids excreted.

At this point it may be stated that the quantities of prunes fed had no significant effect on the acid-base equilibrium. However, because of the conclusions of Bischoff et al. ('34), Fasold ('30) and Shock and Hastings ('34), such a statement might be questioned. The blood  $\text{CO}_2$  combining power of the blood plasma was determined. In no case did the inclusion of prunes cause a variation greater than that which has been observed in healthy normal individuals; Shock and Hastings ('34), Cullen and Earle ('29), Cullen and Robinson ('23), Dodds and McIntosh ('23), and Meyers and Decker ('24). The greatest variation of the average  $\text{CO}_2$  combining capacity during the control and control plus prune diet periods was plus 5 volumes per cent. The larger part of this variation may be attributed to the limit of accuracy of the technic used. In order to determine the variations in the plasma  $\text{CO}_2$  combining capacity at various periods during the day, three men remained on the neutral diet an extra day. Blood samples were drawn at 9.00 A.M. after breakfast, but before the ingestion of prunes, and at 10.00 A.M., 11.00 A.M. and 2.30 P.M. Six, twelve or eighteen prunes were ingested by each individual soon after the blood sample was taken at 9.00 A.M. The variations observed were in accord with the day to day variations of the plasma  $\text{CO}_2$  combining capacity. Even when eighteen prunes were ingested, no significant change in plasma  $\text{CO}_2$  combining capacity occurred. From these results it may be concluded that eighteen prunes (200 gm.) or 120 cc. prune juice have no significant effect on the plasma  $\text{CO}_2$  combining power, in healthy individuals, when included in a neutral diet. Other factors undoubtedly play an important part in maintaining the acid-base equilibrium, but it is beyond the scope of this study to go into these, though the writers are aware that some of these factors may have had some effects on the results obtained.

*Acid diet.* The same subjects that were on the neutral diet were placed on an acid diet, with the exception of subject 7. The data obtained are also given in table 1.

The results obtained were very similar to those obtained on the neutral diet. The urinary pH changes were about the same as those on the neutral diet. The titratable acidity on the acid diet was higher than that of the neutral diet, the organic acids were slightly higher on the acid diet than on the neutral diet. The NH<sub>3</sub> excretion was somewhat less on the acid diet than on the neutral diet. In regard to the CO<sub>2</sub> combining power of the blood plasma, the results obtained were very similar to those of the neutral diet. The inclusion of prunes in the acid diet did not cause a significant lowering in the plasma CO<sub>2</sub> combining capacity. It is rather interesting to note that the averages of the CO<sub>2</sub> combining capacity for each individual during the acid control period and the acid control plus prune period varied within the same range of volume per cent as the same periods of the neutral diet. It may be concluded that eighteen prunes or 4 ounces (120 cc.) of prune juice per day do not produce any significant change in the plasma CO<sub>2</sub> combining power when included in an acid diet equal in acidity to the one used.

*Uncontrolled diet.* Discussion thus far has been restricted to the controlled diets. While controlled diets alone may be sufficient in this type of investigation, it was considered interesting and desirable to supplement these with an uncontrolled diet. To our knowledge, such supplementary work has not been reported. Twelve men of various ages and eating habits were placed on an uncontrolled diet. The ages of the men were as follows: Subject 1, 63 years; 2, 61 years; 3, 57 years; 4, 39 years; 5, 42 years; 6, 23 years; 7, 27 years; 8, 72 years; 9, 23 years; 10, 18 years; 11, 49 years; 12, 23 years. These men were known to have no metabolic disorders. The study of urinary composition and blood alkaline reserve and the effect of prunes on them in such a group of individuals presents interesting data.

The data obtained are given in table 2. Before considering the general data in table 2, a few interesting observations should be mentioned. Subject 10 was found to have taken at least one  $\text{NaHCO}_3$  tablet in the forenoon of the ninth and eleventh days of the diet. On these days the pH values and organic acids of the urine increased and the total acid of the urine decreased. These changes are in accordance with Fasold's findings. The changes in  $\text{NH}_3$  excretion, however, were not significant. Even more interesting was the fact that the plasma  $\text{CO}_2$  combining power did not vary any more than when  $\text{NaHCO}_3$  was not ingested. Subject 1 was found to take  $\text{NaHCO}_3$  every day. The pH values and organic acids of the urine were consistently high and the total acids excreted were consistently low. The results obtained for  $\text{NH}_3$  excreted were also indicative of his habit since they were consistently low. The plasma  $\text{CO}_2$  combining power, however, was but slightly higher than for most other subjects. The averages were lower than several others.

Subject 8 fasted during the fourth, fifth and sixth days. There was a sharp drop in the pH of the urine reaching 5.3 on the third day of fasting. The decrease in plasma  $\text{CO}_2$  combining power was about 10 volumes per cent on the second day. This is to some extent in accordance with the work of Walinski ('26), who found that a 24-hour starvation reduced the alkaline reserve of human blood about 7 per cent and 18 per cent after 48 hours of starvation.

The average diet of these twelve subjects was slightly on the acid side in most instances. The titratable acidity, organic acids and  $\text{NH}_3$  of the urine varied within normal limits. The pH values in all cases except those already discussed were within a range similar to that obtained on the controlled diets. The inclusion of prunes did not consistently change these results in one direction or the other. The plasma  $\text{CO}_2$  combining power changed very little in one direction or the other and these changes were within the range of variations of the men who remained 14 days on the prune free diet. In the diet free of prunes the maximum variation in plasma  $\text{CO}_2$

combining power was from 57.66 to 72.2, or 14.54 volumes per cent. The greatest urinary pH variation was 5.7 to 7.1, or 1.4 pH units. The maximum variation in plasma CO<sub>2</sub> combining capacity for an individual including prunes or prune juice was 53.39 to 69.26, or 15.87 volumes per cent for subject 9, who included prune juice in his diet. The greatest urinary pH variation was 1.87 for the same individual. These figures are given merely to indicate the magnitude of the variations and not to correlate with the ingestion of prunes or prune juice. The extreme variations given for subject 9 all occurred in the diet plus prune juice period.

#### SUMMARY AND CONCLUSIONS

1. The effects of six, twelve and eighteen prunes and 120 cc. (4 ounces) of prune juice on urinary composition and plasma CO<sub>2</sub> combining power when included in neutral, acid and uncontrolled diets were determined. Two men on the uncontrolled diet ingested NaHCO<sub>3</sub> and one man fasted for 3 days during the diet and the effects on the urine and plasma CO<sub>2</sub> combining power were noted.

2. The inclusion of twelve and eighteen prunes in the neutral and acid diets caused an increase in organic acids and a decrease in NH<sub>3</sub> and total acids excreted in most instances. When prunes were included in the diet the urinary pH values dropped in most instances. According to the interpretation of Fasold, the changes in organic acids, total acids, and ammonia were caused by the alkalinity of the prunes, the non-oxidizable benzoic and quinic acids being responsible for a small proportion. The changes occurring when prunes were included in the uncontrolled diet were not significantly different from the changes occurring when prunes were omitted from the uncontrolled diet.

3. The plasma CO<sub>2</sub> combining power was not changed significantly when six, twelve or eighteen prunes or 120 cc. of prune juice were included in the neutral, acid or uncontrolled diets. Variations in plasma CO<sub>2</sub> combining power at various periods during the day before and after the inclusion of prunes in the neutral diet were slight.



4. The variations in plasma  $\text{CO}_2$  combining power were within the normal range as established by various authorities. The greatest variation in plasma  $\text{CO}_2$  combining power and urinary pH occurred on the uncontrolled diet. These variations were not caused by prunes.

5. The changes in urinary composition caused by the ingestion of  $\text{NaHCO}_3$  were in accordance with the findings of Fasold. Fasting caused a drop in urinary pH and plasma  $\text{CO}_2$  combining power.

6. The writers are aware that certain factors as R.Q. and renal function were not taken into consideration; however, they believe since great care was taken picking subjects and conducting the experiments they were sufficiently controlled and show that the inclusion of as much as eighteen prunes (200 gm.) in the diet does not cause a significant variation in one direction or the other in the plasma  $\text{CO}_2$  combining power, but on the uncontrolled diet tend to narrow the range of variation. They also offer an explanation for the changes occurring in urinary composition when this fruit which has a highly alkaline ash is eaten.

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## LITERATURE CITED

- BARR, D. P. 1922 The acid-base equilibrium of the blood following vigorous muscular exercise. *Proc. Soc. Exp. Biol. Med.*, vol. 19, p. 179.
- BISCHOFF, F., W. D. SANSUM, M. L. LONG, AND R. D. EVANS 1932 A comparative study of rabbits maintained on barley or alfalfa. *J. Nutrition*, vol. 5, p. 403.
- BISCHOFF, F., W. D. SANSUM, M. L. LONG, AND M. M. DEWAR 1934 The effect of acid ash and alkaline ash foodstuffs on the acid-base equilibrium of man. *J. Nutrition*, vol. 7, p. 51.
- BLATHERWICK, N. R., AND M. L. LONG 1914 Studies of the urinary acidity. *Arch. Int. Med.*, vol. 14, p. 409.
- 1923 The increased acidity produced by eating prunes and cranberries. *J. Biol. Chem.*, vol. 57, p. 815.
- BRUNTON, C. E., AND C. WILSON 1932 The relation of different foodstuffs to the alkaline tide of the urine. *J. Physiol.*, vol. 74, p. 10.
- CHANNEY, H. S., AND K. BLUNT 1925 The effect of orange juice on calcium, phosphorus, magnesium and nitrogen retention and urinary organic acids on growing children. *J. Biol. Chem.*, vol. 66, p. 829.
- CULLEN, G. E., AND I. P. EARLE 1929 Studies in the acid-base conditions of the blood. Physiological changes in the acid-base conditions throughout the day. *J. Biol. Chem.*, vol. 83, p. 545.
- CULLEN, G. E., AND H. W. ROBINSON 1923 The normal variations in plasma hydrogen ion concentration. *J. Biol. Chem.*, vol. 57, p. 533.
- DENNIG, H. 1931 Über die Grenzen der Arbeits Fähigkeit bei Acidose, Alkalose, und Herzinsuffizienz. *Verh. d. Deutsch. Ges. f. inn. Med.*, 43. Kongress, S. 120.
- DODDS, E. C., AND J. MCINTOSH 1923 Variation of the CO<sub>2</sub> content of blood constituents in relation to meals. *J. Physiol.*, vol. 57, p. 139.
- EIMER, K. 1931 In wie Weit ist eine Beeinflussung des Säuren Basenhaushaltes durch Rohkost möglich? *Verh. d. Deutsch. Ges. f. inn. Med.*, 43. Kongress, S. 163.
- FASOLD, H. 1930 Beiträge zum Säure Basenhaushalt. Über die Bedeutung der Organischen Säuren im Urin bei Vegetabilischen und Animalischer Ernährung. *Zeitschr. f. Kinderheilk.*, Bd. 49, S. 709.
- 1931 a Beiträge zum Säure Basenhaushalt. Über die Bedeutung der Organischen Säuren im Urin. *Zeitschr. f. Kinderheilk.*, Bd. 50, S. 651.
- 1931 b Beiträge zum Säure Basenhaushalt. Über die Assimilierbarkeit der Racemischer Milchsäure und deren Einfluss auf den Säure Basenhaushalt beim Säugling. *Zeitschr. f. Kinderheilk.*, Bd. 51, S. 247.
- 1931 c Über die Bedeutung der Organischen Säuren im Säure Basenhaushalt. *Klin. Wochenschr.*, Bd. 10, S. 961.
- FELLERS, C. R., B. C. REDMAN, AND E. M. PARROT 1933 The effect of cranberries on urinary acidity and blood alkali reserve. *J. Nutrition*, vol. 6, p. 455.
- HAWK, P. B., AND O. BERGEIM 1931 *Practical Physiological Chemistry*. P. Blakiston's Son & Co., Inc., Philadelphia.

- HENDERSON, L. J. 1911 A critical study of the process of acid excretion. *J. Biol. Chem.*, vol. 9, p. 403.
- MICHALOWSKY, E. H. 1930 Alkalireserve und Säure Kost. *Klin. Wochenschr.*, Bd. 9, S. 1505.
- MRAK, E. M., AND W. V. CRUESS 1929 The utilization of surplus prunes. *Univ. Calif. Agr. Exp. Sta. Bull.* 483.
- MYERS, V. C., AND L. E. BECKER 1924 Some variations in the acid-base balance of the blood in disease. *J. Biol. Chem.*, vol. 59, p. 699.
- QUICK, A. J. 1931 The conjugation of benzoic acid in man. *J. Biol. Chem.*, vol. 92, p. 65.
- RADIN, M. J. 1914 A note on the quantity of benzoic acid contained in prunes and cranberries. *Ind. Eng. Chem.*, vol. 6, p. 518.
- RICE, H. A., AND A. H. STEINHAUS 1931 Studies in the physiology of exercise. Acid-base changes in the serum of exercised dogs. *Am. J. Physiol.*, vol. 96, p. 529.
- SALTER, WILLIAM T., CONSTANCE FULTON, AND FRANCES ANGLIER 1931 Studies in calcium and phosphorus metabolism. XI. The calculation of acid-base content of the diet. *J. Nutrition*, vol. 4, p. 1.
- SAMUEL, E. L., AND J. H. KUGELMASS 1930 Comparative studies of the influence of acid forming and base forming diets on the metabolism of rats. *Am. J. Dis. Child.*, vol. 39, p. 687.
- SATWELL, L. G. 1932 a The effect of grapes and grape products on urinary acidity. *J. Nutrition*, vol. 5, p. 103.
- 1932 b The effect of figs and small amounts of raisins on urinary acidity. *J. Nutrition*, vol. 5, p. 519.
- 1933 Effects of pears, peaches, apricots and dried sulfured apricots on urinary acidity. *J. Nutrition*, vol. 6, p. 397.
- SATWELL, L. G., AND M. W. LANE 1933 Comparative effect of tomato and orange juices on urinary acidity. *J. Nutrition*, vol. 6, p. 263.
- SCHWARTZ, H., AND H. DIBOLD 1931 Diätetische Beeinflussung des Säure Basenhaushaltes. *Deutsche med. Wochenschr.*, Bd. 57, S. 1324.
- SHERMAN, H. C., AND J. E. SINCLAIR 1907 The balance of acid-forming and base-forming elements in foods. *J. Biol. Chem.*, vol. 3, p. 307.
- SHERMAN, H. C., AND A. O. GETTLER 1912 The balance of acid-forming and base-forming elements in foods, and its relation to ammonia metabolism. *J. Biol. Chem.*, vol. 11, p. 323.
- SHOCK, N. W., AND A. B. HASTINGS 1934 Variations of the acid-base balance of the blood in normal individuals. *J. Biol. Chem.*, vol. 104, p. 585.
- VAN SLYKE, D. D., AND G. E. CULLEN 1914 A permanent preparation of urease, and its use in the determination of urea. *J. Biol. Chem.*, vol. 19, p. 211.
- 1917 Studies of acidosis. *J. Biol. Chem.*, vol. 30, p. 289.
- VAN SLYKE, D. D., AND N. W. PALMER 1920 Studies of acidosis. The titration of organic acids in the urine. *J. Biol. Chem.*, vol. 41, p. 567.
- WALINSKI, F. 1926 Die Einwirkung des Hungers auf die Alkalireserve im menschlichen Blut. *Klin. Wochenschr.*, Bd. 5, S. 600.

## HUMAN MILK STUDIES

### XVI. VITAMIN D POTENCY AS INFLUENCED BY SUPPLEMENTING THE DIET OF THE MOTHER DURING PREGNANCY AND LACTATION WITH COW'S MILK FORTIFIED WITH A CONCENTRATE OF COD LIVER OIL<sup>1</sup> (A TEST ON RACHITIC INFANTS AND RATS)

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#### FOUR PLATES

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Clinical experience demonstrates that, under present living conditions, neither human milk nor cow's milk can always be relied upon to protect babies against rickets, although rickets is universally less frequent among nursing infants than among bottle-fed babies. Despite the fact that specific treatment for the prevention and cure of this disease has been known for several years and many antirachitic agents are not only in the hands of the physicians and public health workers, but are being distributed widely to the layman, recent observations have shown that 26 per cent of breast-fed and 56 per cent of the formula-fed babies living in our city have rickets (Barnes, Brady and James, '30).

Laboratory biological tests have shown that ordinary breast milk is a poor source of vitamin D, since a supplement of 20 cc. of woman's milk daily did not protect a rat of usual age and weight from rickets (Hess and Weinstock, '27); even 25 to

<sup>1</sup> The National Oil Products Company, Harrison, New Jersey was kind enough to furnish the fortified cow's milk for the mother and to pay for the breast milk used in this study.

40 cc. of pooled human milk of women on the average American dietary failed to produce healing in rachitic rats (Outhouse, Macy and Brekke, '28). This fact that mother's milk contains very little of the anti-rachitic factor when given to the rat as the universal experimental test animal for rickets becomes of greater importance when it is borne in mind that there is an essential difference between the reaction of the infant and the rat in regard to response to unitage of vitamin D (Barnes, Brady and James, '30).

The biological potency of breast milk can be enhanced up to a specific physiological limit by the addition of a concentrated source of the vitamin B complex (Donelson and Macy, '34) and vitamin A (McCosh et al., '34) to the average dietary and that mother's milk produced during an inadequate dietary regime may fall short of meeting the optimal concentration of vitamin A in milk, and, therefore, the physiological needs of the infant (Macy, et al., '28; Thatcher and Sure, '32; Kennedy, et al., '23). It does not seem improbable that woman's milk may be fortified in respect to vitamin D by the administration of an antirachitic substance under propitious dietary conditions. It is desirable to have as complete an understanding as possible, based on investigations in the clinic as well as in the laboratory, of the relationship of woman's milk, produced under dietary supplements of the current anti-rachitic substances, to the development and cure of rickets.

There is a tendency at present to exaggerate the protective value of breast milk against rickets and dental disturbances when the mother takes in her daily diet any one of a number of vitamin D preparations. Clinical proof does not exist for some of the present day advertising in this regard. This may be harmful to the child, as it has led mothers to believe that it is unnecessary for the nursing infant to take directly supplementary additions of vitamin D.

There is apparently a variation in the degree of individual response of women to specific medication as well as to different types of antirachitic treatments for it is shown that babies do not enter the world with an equal susceptibility to rickets

(Hess, '29). Furthermore, in clinical tests woman's milk can be endowed adequately with vitamin D by exposing the nursing woman to ultra-violet light as demonstrated by the healing of rickets in infants, whereas a similar enhancement of human milk cannot be brought about by giving to nursing mothers one tablespoonful of cod liver oil in addition to a satisfactory diet (Gerstenberger et al., '27).

The present report is a part of a series of studies on the effectiveness of diets rich in vitamins on the nutrition of the woman during pregnancy and lactation, on the biological potency of the milk secreted and on the extent to which the vitamins are transmitted to the young through the placenta and through the milk.

#### EXPERIMENTAL

In view of the fact that women respond differently to various types of antirachitic treatment, in this study of the vitamin D potency of mother's milk as influenced by supplementing the diet during pregnancy and lactation with cow's milk fortified with a concentrate of cod liver oil, a woman<sup>2</sup>, who had demonstrated herself previously to have a capacity for a large milk flow, was chosen as the source of supply for the milk used in this investigation. This assured an experimental milk of a constant source and quality for both the control and experimental babies. Her diet over a period of detailed metabolic studies throughout two former reproductive cycles was adequate according to accepted dietary standards. In addition to the well-selected diet, this mother chose to take continuously for this study from the nineteenth week of pregnancy and throughout lactation 2 quarts of milk fortified with a cod liver oil concentrate containing a total of 300 units of vitamin D per day.<sup>3</sup>

<sup>2</sup> A continuous acid-base mineral balance on this mother from the nineteenth week of pregnancy through eight weeks of lactation will be reported in the near future.

<sup>3</sup> The vitamin D milk furnished the mother was assayed by C. A. Hoppert, Ph.D., Department of Chemistry, Michigan State College.

The mother's own infant served as the control and the three experimental babies were selected from breast-fed infants who had developed severe clinical rickets as shown by roentgen examinations and by determinations of the blood calcium and phosphorus. The control baby had monthly x-rays from birth through the 8th month, whereas the experimental rachitic babies had roentgenograms and bloods taken immediately preceding the introduction of the experimental milk and at frequent intervals thereafter.

#### RESULTS AND DISCUSSION

Table 1 and plate 1 demonstrate that a mother who continuously consumed from the nineteenth week of pregnancy through 8 months of lactation a generous mixed dietary enriched daily with 2 quarts of milk to which 300 units of vitamin D in the form of a concentrate of cod liver oil were added, was able to bear and rear an infant (J. R.) who showed no clinical signs of rickets during the first 8 months of life, including the rachitic season. Adequate protection was given to the infant, therefore, either by a transfer of vitamin D through the placenta or the milk, or both. In addition, and perhaps of even greater significance, a more favorable provision for bone growth was furnished by a superior maternal mineral metabolism. According to the results obtained during a continuous mineral metabolic balance of the mother during her present reproductive cycle, she was retaining more than twice the average amount (Macy and Hunscher, '34) of bone building constituents.<sup>4</sup>

On the contrary, three colored breast-fed babies who had severe clinical rickets as shown by roentgen examinations and by chemical determinations of blood calcium and phosphorus showed no improvement when placed for periods varying from 11 to 43 days on 30 to 32 ounces daily of breast milk produced by the mother in this study (table 1 and plates 2 to 4). Insufficient amounts of vitamin D were transmitted through the breast milk alone to induce healing of rickets in these three

<sup>4</sup> See footnote 2, page 649.







babies although the same milk simultaneously prevented the occurrence of rickets in the mother's own baby. The vitamin D potency and the ineffectiveness of this particular breast milk that was produced under superior dietary conditions was further verified by appropriate curative tests on rachitic rats in which it was shown to contain little more than a trace of vitamin D.<sup>5</sup>

TABLE 1

*Blood and x-ray studies on rachitic infants receiving breast milk from a mother whose diet was supplemented with cow's milk fortified with 300 units of cod liver oil*

SUBJECT	AGE AT BEGINNING	DATE	WEIGHT	NUMBER OF DAYS ON NEW BREAST MILK	TOTAL BREAST MILK TAKEN	CALCIUM	PHOSPHORUS	Ca X P PRODUCT	X-RAY DIAGNOSIS OF RICKETS
Control	Wk.		Kg.		Oz.	Mg./100 cc.	Mg./100 cc.		
J. R. (white)	19	11/ 9/33	6.98	....	....	....	....	....	Normal
	35	3/ 5/34	9.00	....	3920	....	....	....	Normal
L. C. (colored)	53	11/17/33	8.82	0	....	9.82	2.32	22.8	+++
		11/21/33	....	4	....	9.63	2.21	21.3	....
		11/28/33	....	11	....	10.09	2.64	26.6	....
		12/12/33 <sup>1</sup>	9.36	23	832	9.26	2.66	24.6	+++
V. W. (colored)	21	1/12/34	6.44	0	....	9.82	2.82	27.7	++++
		....	....	12	....	10.20	2.90	30.0	....
		2/16/34	7.29	24	928	8.27	3.63	30.0	++++
J. O. (colored)	36	1/24/34	9.00	0	....	8.43	3.57	30.0	+++
		2/ 6/34	9.08	13	....	10.26	....	....	....
		3/13/34	8.90	43	1296	10.50	3.47	36.4	++++

<sup>1</sup> After 1 month healing was evident and complete in 2 months on evaporated milk to which 150 units of cod liver oil concentrate had been added.

When the same concentrate of vitamin D was given directly to the infants in a cow's milk formula, healing of rickets was evident in less than a month and was complete in 2 months

<sup>5</sup> The biological tests on the breast milk were kindly conducted by Henry C. Sherman, Ph.D., and Theodore F. Zucker, Ph.D., both of Columbia University, New York City.

(plates 2 to 4). Exclusive of the advantage to the mother and her own infant, direct administration of vitamin D to the rachitic infant is more effective than the indirect method via breast milk. Of preeminence is the fact that the infant, whose mother's diet was known to be superior not only in vitamin D, but in minerals and proteins of excellent quality during both pregnancy and lactation, was able to build normal bone up to the eighth month of life with no direct administration of vitamin D.

#### SUMMARY

A woman whose diet was superior in quality and, in addition, was fortified with 2 quarts of cow's milk daily to which 300 units of a vitamin D concentrate of cod liver oil were incorporated, was unable to secrete a breast milk that was sufficiently enriched with vitamin D to heal rickets in three colored breast-fed infants or in experimental rachitic rats. Her own breast-fed baby, however, showed no signs of rickets throughout the investigation.

#### LITERATURE CITED

- BARNES, D. J., M. J. BRADY, AND E. M. JAMES 1930 The comparative value of irradiated ergosterol and cod liver oil as a prophylactic and antirachitic agent when given in equivalent dosage according to rat units of vitamin D. *Am. J. Dis. Child.*, vol. 39, p. 45.
- DONELSON, E. G., AND I. G. MACY 1934 Human milk studies. XII. The vitamin B and vitamin G content before and during maternal consumption of yeast. *J. Nutrition*, vol. 7, p. 231.
- GERSTENBERGER, H. J., J. I. HARTMAN, AND D. N. SMITH 1927 The antirachitic value of human milk. *Calif. and Western Med.*, vol. 27, p. 40.
- HESS, A. F. 1929 Rickets including osteomalacia and tetany. Philadelphia: Lea & Febiger.
- HESS, A. F., AND M. WEINSTOCK 1927 A study of the antirachitic factor in human and cow's milk. *Am. J. Dis. Child.*, vol. 34, p. 845.
- KENNEDY, C., L. S. PALMER, AND F. W. SCHLUTZ 1923 The vitamin content of breast milk. *Tr. Am. Ped. Soc.*, vol. 35, p. 26.
- MACY, I. G., J. OUTHOUSE, AND H. A. HUNSCHER 1928 The variability in vitamin content of human milk. *J. Home Econ.*, vol. 20, p. 897.
- MACY, I. G., AND H. A. HUNSCHER 1934 An evaluation of maternal nitrogen and mineral needs during embryonic and infant development. *Am. J. Obstetrics and Gynecology*, vol. 27, p. 878.

- MCCOSH, S. S., I. G. MACY, H. A. HUNSCHER, B. N. ERICKSON, AND E. DONELSON 1934 Human milk studies. XIII. Vitamin potency as influenced by supplementing the maternal diet with vitamin A. *J. Nutrition*, vol. 7, p. 331.
- OUTHOUSE, J., I. G. MACY, AND V. BREKKE 1928 Human milk studies. V. A quantitative comparison of the antirickettic factor in human milk and cow's milk. *J. Biol. Chem.*, vol. 78, p. 129.
- THATCHER, H. S., AND B. SURE 1932 Avitaminosis. III. Pathologic changes in tissues of the albino rat during the early stages of vitamin A deficiency. *Arch. Path.*, vol. 13, p. 757.

VITAMIN D POTENCY OF HUMAN MILK  
DONALD J. BARNES AND OTHERS

J. R.



9/23/33

PLATE 1

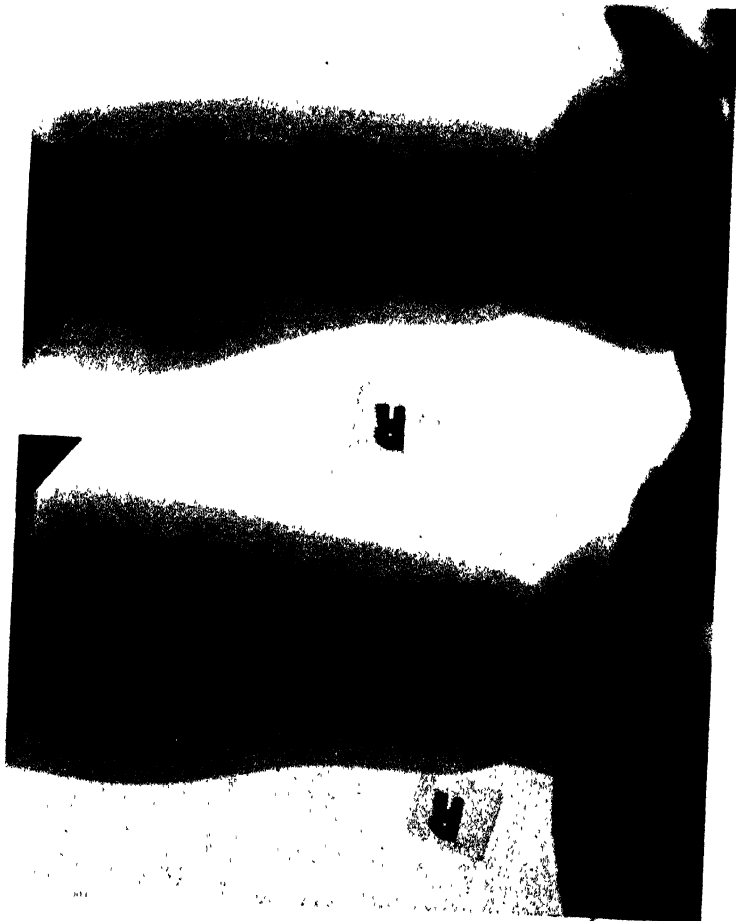


2/13/34

VITAMIN D POTENCY OF HUMAN MILK  
DONALD J. BARNES AND OTHERS

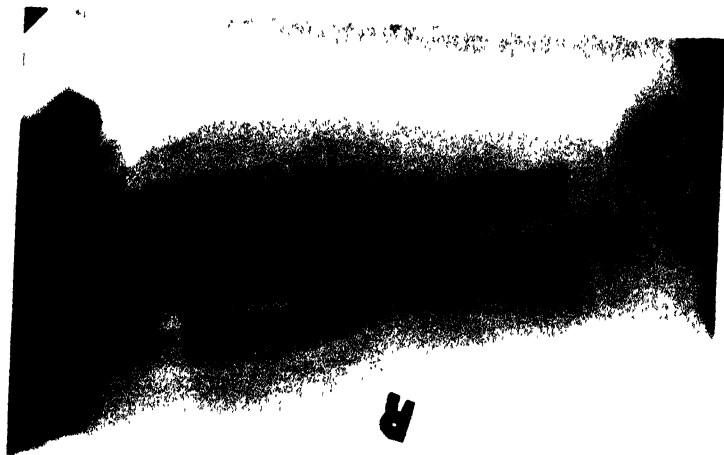
L. C.

PLATE 2



11/16/33

12/12/33



3/1/34

VITAMIN D POTENCY OF HUMAN MILK  
DONALD J. BAERNES AND OTHERS

V. W.



2/6/34



1/11/34

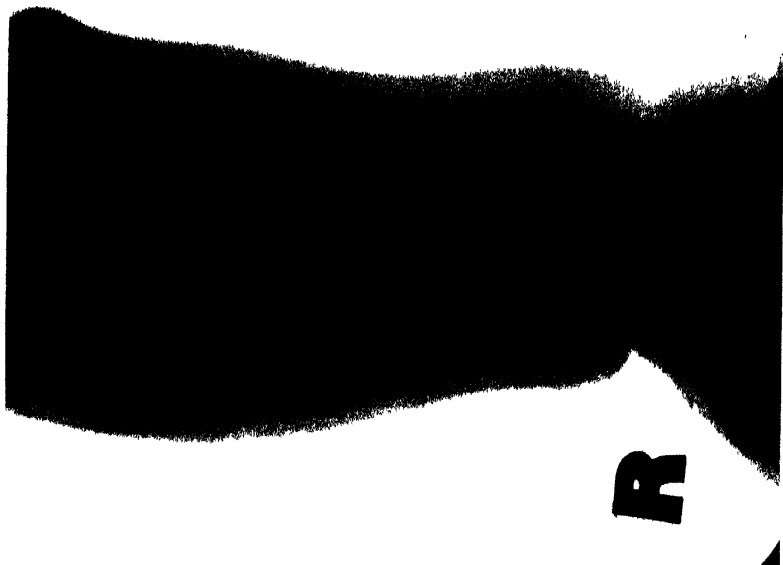
VITAMIN D POTENCY OF HUMAN MILK  
DONALD J. BARNES AND OTHERS

J. O.

PLATE



1/24/34



2/6/34





# INORGANIC SALTS IN NUTRITION

## IX. CORRELATION BETWEEN SUPPRESSED GROWTH AND THE DEVELOPMENT OF POLYCYTHEMIA INDUCED BY FEEDING A RATION POOR IN SALTS <sup>1</sup>

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### TWO CHARTS

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It has recently been demonstrated (Swanson and Smith, '32 a, b) that it is possible to induce a severe and true experimental polycythemia in the rat by greatly reducing the inorganic residue of an otherwise adequate ration when casein serves as the dietary protein. Upon such a regimen the number of red cells in the blood of the experimental animals increased some 60 per cent in 3 months, whereas the increment observed in the blood of the normal rats receiving a sufficient quantity of a favorable salt mixture in the diet was only 27 per cent. Furthermore, the erythrocytes were subnormal in size and, in contrast to the usual picture in clinical poly-

<sup>1</sup> Some of the data reported in this paper are taken from a dissertation presented by Pearl P. Swanson in partial fulfillment of the requirements for the degree of doctor of philosophy, Yale University, 1930. Other parts represent findings in the extension of the study at Iowa State College. Grateful acknowledgment is made for the technical assistance rendered by Ernestine Frazier and Gladys Timson, Iowa State College.

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The preceding article of the series may be found in the *J. Biol. Chem.*, vol. 105 p. 181.

<sup>2</sup> Alexander Brown Coxe Fellow in Physiological Chemistry, Yale University, 1929-1930.

cythemia, the concentration of hemoglobin in the blood was subnormal.

In the early experiments, the polycythemia occurred in practically every animal of a fairly large group receiving the defective ration. Later, however, when the investigation was extended, it was found in many instances that only a mild polycythemia had been induced and that in certain cases, the blood picture was entirely normal. The present report deals with certain correlations bearing on these observations.

#### SUPPRESSION OF GROWTH AND THE INCIDENCE OF POLYCYTHEMIA

Rats of an average body weight of 160 gm. were employed in the studies in order to obtain sufficient quantities of blood for analytical purposes. Therefore, due to the stunting effects of the diet, a preliminary period of growth upon an adequate ration was necessary before it was possible to inaugurate the low salt feeding (Swanson and Smith, '32a). Accordingly, the experimental ration was not offered until the rats had attained a body weight of 120 gm. After maintenance upon this diet for 3 months, the adult rats were as definitely polycythemic as were very young animals after 1 month (Smith and Schultz, '30). It was noted, however, that with the larger rats the administration of the defective ration did not produce the immediate suspension of growth that occurred when the low salt diet was given to the newly-weaned animals (Winters, Smith, and Mendel, '27). An analysis of the growth performance of the rats used in the present study showed that rats varied in their response to the deficient diet. Although all weighed 120 gm. at the initiation of the experiment, some individuals attained a final body weight of about 160 gm.; others, 180 gm.; and still others, 200 gm. The entire group of experimental animals (sixty-five rats), classified according to their body weight, fell into one or the other of the three weight groups indicated above. The rats in group I ( $158 \pm 12.4$  gm.<sup>\*</sup>) grew at a retarded rate for a period of 32 days, attaining an average weight of 158 gm.

\* Standard deviation.

No further increments in growth occurred thereafter and the body weight was maintained at this level for the remainder of the experimental period. The individuals of group II also increased in weight, but the subnormal growth extended over a longer interval than in the case of the animals of group I (56 days vs. 32 days). Growth then ceased and the body weight ( $183 \pm 8.8$  gm.) remained constant for the next 32 days, after which the experiment was terminated. The growth of the animals in group III was never completely inhibited. They grew slowly for nearly the entire experimental period. During the last 2 weeks they showed a tendency to lose weight. However, the average body weight at the end of the period was still high, i.e.,  $199 \pm 7.8$  gm. A biometric interpretation<sup>4</sup> of the growth of the three groups of rats receiving the ash-poor ration is recorded in chart 1.

Inasmuch as the polycythemia described by Smith and Schultz ('30) occurred after a period of maintenance of constant weight upon the low salt diet, it seemed likely that the varied growth performance of our rats might be related to the different degrees of severity of polycythemia observed. It was found that rats maintaining an average body weight of approximately 158 gm. for 58 days (group I) had an average of twelve million red cells per cubic millimeter of blood; those holding a body weight of 180 gm. for 32 days (group II), ten and seven-tenths million; and those that grew for the entire experimental period (group III), nine and nine-tenths million. Thus, it is evident that the degree of polycythemia is correlated with the suppression of growth of the test animal fed the salt-poor ration. Whenever growth occurs at a constant, though minimal, rate, the blood picture remains normal in spite of the imposed dietary deficiency (group III, chart 1). In order to induce an increment in the number of red cells during a 3-month period that is twice the normal, an inhibition of growth must occur early in the experimental history (after

<sup>4</sup> The solid middle lines show the mean growth curves of the groups. The standard deviations from the average weight of the respective intervals are represented on each curve by the dotted line on each side of the average.

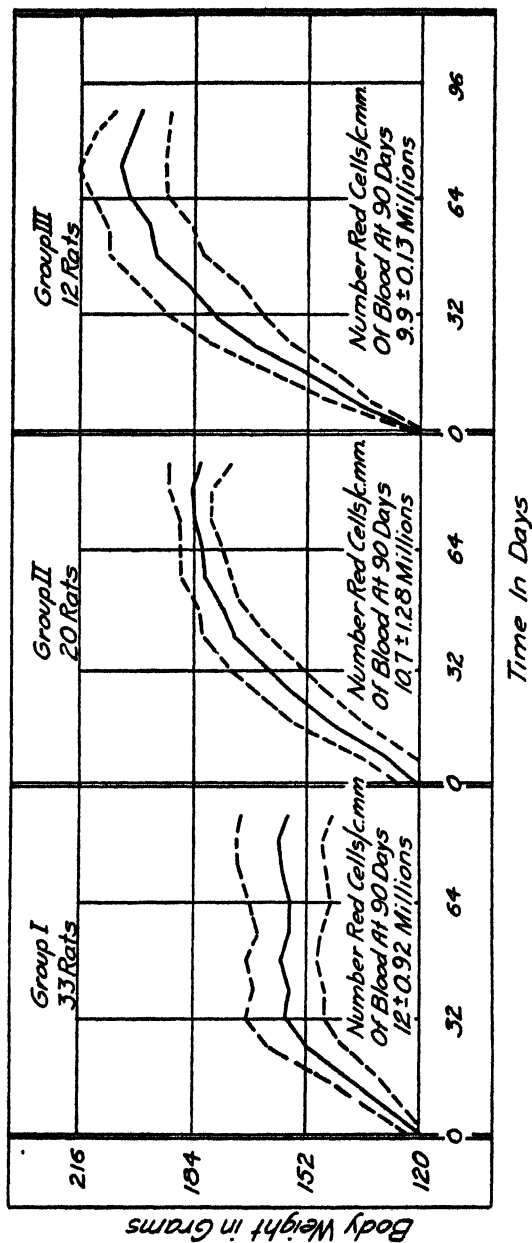


Chart 1 Variation in the growth response and the erythrocyte count of a group of sixty-five rats placed upon a low salt ration upon the attainment of a body weight of 120 gm.

1 month) with maintenance of weight thereafter (group I, chart 1).

The importance of a protracted period of maintenance of constant weight for the production of the polycythemia is illustrated in the results obtained in the continuation of the studies at Iowa State College when a different strain of animals was used (Wistar, strain B). A comparison of curves A and B in figure I, chart 2, shows that although the Iowa rats stunted near the desired level of 160 gm., constant weight (defined as  $x \pm 10$  gm.) was maintained for a period only approximately one-half as long as was the case in the Yale rats. The average erythrocyte concentration in the blood of these rats was ten and seven-tenths million per cubic millimeter, a value only slightly above normal (nine and seven-tenths million). Three rats were chosen from this group and maintained upon the defective diet for another month. The red cell count rose from values of ten and three-tenths, ten and five-tenths and nine and eight-tenths million per cubic millimeter of blood at the end of 3 months to twelve and one-tenth, eleven and nine-tenths, and ten and nine-tenths million, respectively, when the experimental ration was given for this additional time. A representative case is depicted in figure II, chart 2. This experiment suggests that if a rat responds to the low salt diet in such manner that the body weight is not held at a constant level for at least 7 to 8 weeks, the experimental period must be lengthened. The practical drawback of this situation from the point of view of experimentation is at once apparent.

However, with a slight modification of experimental technic, it was possible to obtain the same average type of curve in the second laboratory that was typical of the growth of the rats grown at Yale, without lengthening the time of the experiment. Furthermore, the polycythemia was just as severe. The effects induced by the feeding of the low salt food were duplicated despite the fact that the animals employed were taken from a colony of a new strain maintained upon a different stock ration. Instead of keeping the animal upon the

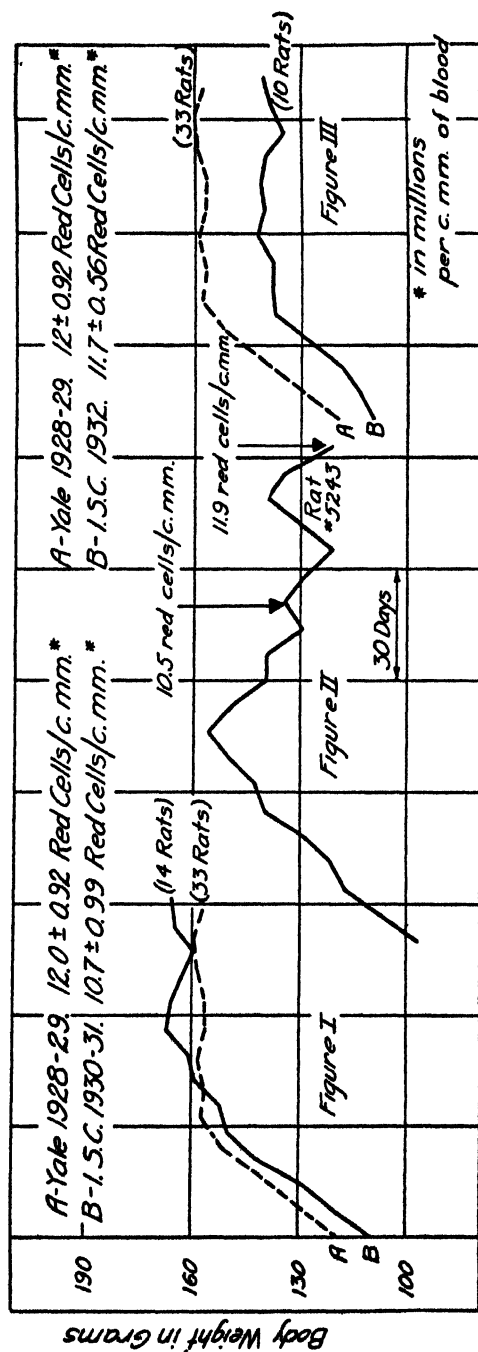


Chart 2 The relation between the length of time of maintenance of constant weight and the number of erythrocytes in the blood of rats fed a diet poor in salts.

adequate stock ration until a body weight of 120 gm. (the standard initial weight of the Yale animals) had been attained, the pre-experimental period was arbitrarily cut to 9 days. With this treatment, the average initial weight at the beginning of the experiment was only 104 gm. Furthermore, the rats grew only for 1 month and thereafter maintained a constant body weight for the remaining time (56 days). The average number of the erythrocytes in the blood of this group of rats was eleven and seven-tenths million per cubic millimeter (fig. III, chart 2), thus approximating the average number in the blood of the group studied in the Yale laboratory which had the same characteristic type of growth curve. The curves obtained in the two laboratories seem to indicate that a definite period of inhibited growth is more closely correlated with the severity of the induced polycythemia than is the level at which such inhibition occurs. It is to be emphasized at this point, however, that suppressed growth per se is not responsible for the polycythemic blood. For instance, retardation of growth brought about either by the limitation of food energy (Smith and Schultz, '30; Swanson and Smith, '32 a) or by the restriction of lysine (Gross, '34) is not accompanied by the marked increase in the number of erythrocytes discussed in the foregoing paragraphs. The retarded growth must be induced by a lack of the mineral salts in the diet if polycythemia is to occur.

From the above experiments, it appears that the investigator interested in the study of the physiological adjustments produced by the elimination of the dietary salts will find it necessary to study the reaction of his particular strain of animals to this specific nutritive deficiency. Furthermore, coprophagy must be prevented in so far as possible and utmost cleanliness of cages and accessories must be maintained if adequate suppression of growth is to occur. We have found that a very narrow margin exists between the quantity of inorganic materials which will restrict growth and the amount which will permit it.



Along with the characteristic suppression of growth, changes occurred in the general appearance of the rat. When the animal had developed a severe polycythemia it was decidedly marasmic. This was not true when the polycythemia was mild; the animal then looked nearly normal. It was surprising to note to what a slight degree the dietary deficiency was reflected in the appearance of the rat when polycythemia did not develop. When a definite polycythemia was present, the body was angular, the hair rough and often absent in certain parts of the body. The standing posture was high and angular. The rat frequently showed signs of extreme nervousness and hyperactivity. Evidence of a functional disturbance of the neuromuscular system occurred late in experimental history. Often the rat lost its sense of equilibrium, and in some instances, severe paralysis of legs and sphincters developed. The snouts were dirty and bloody. The eyes, ear, and paws became less and less pink and near the end of the experimental period were gray-white. The tail was dry and scaly. The leg bones and feet were long and large in proportion to the size of the animal. A severe diarrhea appeared almost immediately upon the administration of the low salt food and continued for approximately 3 weeks. Even though the intensity of the diarrhea subsided, the feces remained somewhat softer than normal throughout the entire experimental period.

At autopsy, there was extreme paucity of visceral fat. The cecum was often distended, and enlargement of the kidneys and adrenals was observed. Although the kidneys were frequently of a gray color with a mottled appearance, other visceral organs appeared normal. The lungs rarely showed atelectases or infection—a finding in harmony with the suggestion of Moïse and Smith ('30) that the active rat is less likely to develop bronchial obstruction and subsequent atelectasis than is the sluggish animal.

The restriction of growth, the change in body contour, and the dystrophic growth of the long bones as well as the depletion of the skeleton of ash constituents were all revealed in

x-ray pictures of rats fed the low ash diet. Extreme osteoporosis, not rickets, was indicated, shown also by difficulty experienced in handling the animals without fracturing a leg bone. The molars were often loose and the incisors broken. There is a decided uniformity in the picture presented by the rats reared upon a dietary regimen characterized by nearly a complete absence of the inorganic salts and, so far as we are aware, it is not like any thus far described.<sup>5</sup>

#### CONCLUSIONS

1. Unless the growth impulse can be inhibited in the mature albino rat by the feeding of a salt-poor ration with casein as the protein, a polycythemia is not produced.

2. Constant weight following a short period of sub-normal growth must be maintained for at least 56 days in order to induce a pronounced increment in the number of red cells during the experimental interval.

3. The polycythemia is accompanied by a characteristic marasmic bodily state.

#### LITERATURE CITED

- GROSS, G. D. 1934 Unpublished observations, Department of Physiological Chemistry, Yale University, New Haven.
- MOÏSE, T. S., AND A. H. SMITH 1930 Atelectasis in the pathogenesis of abscess of the lung. *Arch. Int. Med.*, vol. 45, p. 92.
- SMITH, A. H., AND R. V. SCHULTZ 1930 Effects of diet poor in inorganic salts on certain organs and blood of young rats. *Am. J. Physiol.*, vol. 94, p. 107.
- SWANSON, P. P. 1930 Certain effects induced by a ration deficient in inorganic constituents. Dissertation, Yale University.
- SWANSON, P. P., AND A. H. SMITH 1932 a Inorganic salts in nutrition. IV. Changes induced in the blood by a ration deficient in inorganic constituents. *J. Biol. Chem.*, vol. 98, p. 479.
- 1932 b Inorganic salts in nutrition. V. Progressive changes in the blood of rats maintained upon a ration poor in inorganic salts. *J. Biol. Chem.*, vol. 98, p. 499.
- WINTERS, J. C., A. H. SMITH AND L. B. MENDEL 1927 The effects of dietary deficiencies on the growth of certain body systems and organs. *Am. J. Physiol.*, vol. 80, p. 576.

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<sup>5</sup>The extent of the deficiency in inorganic salts is indicated by data in the next paper of this series (Smith, A. H., and P. K. Smith. 1934. *J. Biol. Chem.*, vol. 107, p. 681.



# THE DERIVATION OF FACTORS FOR COMPUTING THE GASEOUS EXCHANGE AND THE HEAT PRO- DUCTION IN THE METABOLISM OF CASEIN BY THE ALBINO RAT <sup>1</sup>

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In connection with recent experiments conducted in this laboratory on the specific dynamic effect of casein, the question arose as to what factors should be used in computing from the amounts of casein metabolized, as measured by the excretion of urinary nitrogen, the heat, CO<sub>2</sub> and the O<sub>2</sub> equivalents. The factors which are commonly employed for computing the respiratory exchange and the heat production of protein metabolism are those calculated by Loewy ('11) which represent the metabolism of meat protein in the dog. Loewy has also reported in the same publication somewhat different factors for milk protein which were calculated on the basis of work of Rubner (1898) and Rubner and Heubner (1898) with human beings. Since in the casein experiments cited above the albino rat served as the experimental animal, it was thought desirable to determine the factors to be used directly with these animals. The results of these endeavors are recorded below.

Nitrogen, carbon and energy balance experiments were conducted with a group of five rats, each weighing about 100 gm. and receiving 3.8 gm. of commercial casein daily.

<sup>1</sup> Publication authorized by the director of the Pennsylvania Experiment Station January 15, 1934, as technical paper no. 631.

The casein used was found, on analysis, to contain 7.31 per cent moisture, 4.16 per cent ash, 13.18 per cent nitrogen, 47.24 per cent carbon, 0.54 per cent ether extract and 5.097 calories per gram.

The rats were kept in individual cages and were fed twice daily, receiving the experimental diet in two equal portions, for a period of 6 or 7 days. The first 3 or 4 days were preliminary, while the last 3 days constituted the excreta collection period. During the collection period the rats were kept in metabolism cages which were very similar to that described by Levine and Smith ('25), and the feces and urine were collected separately.

The urine and the feces were analyzed for total nitrogen, carbon and energy, the urine of each rat being analyzed separately. The quantities of feces collected for the individual rats were small, and for this reason they were composited, after air drying, into one sample for analysis.

The average results of the balances of matter and energy, and the derivation of the respiration and energy factors for casein, are shown in table 1.

The data of table 1 are for the most part self-explanatory. All the data in the upper part of the table are as experimentally determined. The values given for energy and carbon per 100 gm. of pure casein are based directly on the analysis of the casein used, assuming that the effects on these values of the small quantities of fat and carbohydrate contained in the casein are negligible. In fact, it was calculated, on the basis of the analysis of the material that the error involved by this assumption is less than 1 per cent. The values given for nitrogen, carbon and energy in feces and urine per 100 gm. of pure casein are computed from the determined values, in the upper part of the table, in proportion to the nitrogen content of the casein.

No experimental determinations were made of the intramolecular hydrogen or oxygen content in either the casein or the excreta, and these values were derived as indicated. The computation of the hydrogen and oxygen content of the



excreta probably involves a slight error, inasmuch as this computation assumes that the excretion products, in relation to nitrogen, are the same as those of meat proteins in the dog. However, the writers are not aware of any better data than those used upon which to base this computation.

Of the total nitrogen ingested, 3.4 per cent appeared in the feces and 95.6 per cent appeared in the urine, leaving a positive balance of only 1 per cent.

Of the total calories ingested, 3.4 per cent appeared in the feces and 15.8 per cent appeared in the urine. In other words, 96.6 per cent of the calories of the casein were absorbed and only 80.8 per cent were metabolized. It should perhaps be stated here that the energy of the urine was corrected, as usual, to nitrogen equilibrium, the correction being very small on account of the very small positive nitrogen balance.

Calculated on the basis of pure casein, 3.6 per cent of the total calories appear in the feces and 16.6 per cent in the urine, 96.4 per cent being absorbed and 79.8 per cent metabolized.

The values obtained for  $O_2$  required, and for  $CO_2$  and heat produced, in the metabolism of protein, as casein, are 6.67 liters, 5.47 liters, and 30.59 Calories, respectively, per gram of urinary nitrogen. The respiratory quotient is 0.821, and the calorific value of respiratory  $O_2$  is 4.586 Calories per liter. Loewy's ('11) factors for milk protein are 5.0 liters  $O_2$ , 4.6 liters  $CO_2$  and 27.0 Calories per gram of nitrogen in the urine. His factors for meat protein are 5.94 liters  $O_2$ , 4.75 liters  $CO_2$  and 26.51 Calories per gram of urinary nitrogen.

Rapport ('24) has calculated the following factors for gelatin: 1 gm. of N in the urine = 4.70 liters  $O_2$ , 3.92 liters  $CO_2$  and 22.08 Calories.

Rapport's observation that the calculation of the metabolism of proteins other than meat on the basis of factors for meat protein involves a relatively small error in the total heat production is confirmed by the writers. This can be explained by the fact that the calorific value of a liter of re-

spiratory  $O_2$  in the metabolism of protein does not differ much from the calorific value of  $O_2$  in the metabolism of fat, or of a mixture of fat and carbohydrate. It is well to point out, however, that the percentage contribution of the protein metabolism to the total heat production may be appreciably affected if the calories per gram of urinary nitrogen for a particular protein differ considerably from the corresponding value for meat.

The factors for casein determined in table 1 differ markedly from Loewy's factors for milk protein. In view of the fact that Loewy's figures for  $CO_2$  and  $O_2$  give a respiratory quotient as high as 0.92, the writers feel justified in suspecting that some of Loewy's figures are in error. This suspicion is strengthened by the fact that by dividing the calorific value of a liter of respiratory  $O_2$  (4.66 Calories), given by Loewy, into the calories per gram of urinary nitrogen (27.0) the result obtained, which should represent liters of  $O_2$  per gram of urinary nitrogen, is 5.8 instead of the figure 5.0 which appeared in Loewy's report.

#### SUMMARY

Balances of nitrogen, carbon and energy were determined with five albino rats receiving casein exclusively in quantities sufficient approximately to meet the energy requirements.

Of the total calories of the casein ingested 96.6 per cent were found to be digested, and 80.8 per cent were found to be metabolizable.

The following factors were determined for computing the gaseous exchange and the heat production in the metabolism of casein: 1 gram of urinary nitrogen = 6.67 liters of respiratory  $O_2$ , 5.47 liters of  $CO_2$  and 30.59 Calories.



## LITERATURE CITED

- LEVINE, H., AND A. H. SMITH 1925 A cage device for the study of ketosis and nitrogen metabolism in small animals. *J. Lab. and Clin. Med.*, vol. 11, p. 3.
- LOEWY, A., AND C. OPPENHEIMER 1911 *Handbuch der biochemie des Menschen und der Tiere*, Jena, Bd. 4, Teil 1, S. 279.
- MATHEWS, A. P. 1930 *Physiological Chemistry*, 5th ed. William Wood & Co., New York.
- RAPPORT, D. 1924 The relative specific dynamic action of various proteins. *J. Biol. Chem.*, vol. 60, p. 500.
- RUBNER, M. 1898 *Milchnahrung beim Erwachsenen*. *Zeitschr. für Biol.*, Bd. 36, S. 56.
- RUBNER, M., AND O. HEUBNER 1898 *Die natürliche Ernährung eines Säuglings*. *Zeitschr. für Biol.*, Bd. 36, S. 1.

# THE EFFECT OF VITAMIN A DEFICIENCY ON THE CONCENTRATION OF THE BLOOD LIPIDS OF ALBINO RATS

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The work in this paper is an investigation of fatty acids, lecithin, and cholesterol of the blood of rats when the diet is deficient in vitamin A.

Fat metabolism in avitaminosis has been studied quite extensively by Kazuo Asada ('23 a). He used young rats for his experimental animals, and fed them a vitamin free diet, consisting of wheat protein, starch and dried carrots autoclaved, a salt mixture and distilled water. In his first published report Asada found an increase of blood fat accompanied by a decrease of the total fat of the whole body. It was necessary, therefore, to assume an increased fat combustion and a decreased fat storage. These results about the behavior of fats in avitaminosis led to the knowledge "That there was a disturbance in the movement of the fat, and an increased transport of the body and food fats to the places of combustion."

In Asada's ('23 b) second report emphasis is placed upon the blood as a means of fat transportation. The tabulated results show that "The amount of fat in the blood of avitaminotic animals is somewhat higher than that of normal animals. The cholesterol content of the blood goes parallel with the total fat. . . . In the last stages of avitaminosis there is a decrease in the blood fat which is due to the fact that the

fat depots are exhausted and do not give any more to the blood." In this third report Asada ('23 c) again announces that, the fat and cholesterol content of the bodies of avitaminotic animals decrease while the concentration of the fat and cholesterol of the blood increase.

Fukunda ('30) reported on the change of the "Fat content of the blood in animals fed with a vitamin-poor diet." "In various avitaminoses with animals and birds there is always an increase in the fat content of the blood, the increase being sometimes appreciable even before the manifestations of the symptoms. . . . The character of increased fat is different with different types of avitaminosis. . . . In animals affected with avitaminosis the fat metabolism is deranged."

Wendt ('28), in summarizing his experiments on "Lipid metabolism studies on starving animals," states that, "The lipid phosphorus and cholesterol in the peripheral blood of fasting dogs increase the first few days of the fast and then decrease to sub-normal values as the fasting continues."

Collazo and Bosch ('23) used guinea pigs and dogs for their experimental animals. They concluded that in the course of avitaminosis there was an increase in the total blood fat which decreased at the end of the disease without reaching the normal value. The cholesterol content of the blood went parallel with the fat values. The phosphatide content of the blood was normal at the beginning of avitaminosis and sometimes decreased in the later phases of the disease.

#### EXPERIMENTAL

Albino rats from 50 to 75 days of age were used in this experiment. The animals were arranged in litter mate triads of the same sex, and as nearly comparable in weight as possible. When weight dissimilarity was unavoidable the heaviest animal was chosen for the vitamin A deficient member of the triad, the lightest one was made positive control and the one of medium weight was chosen for the calorie control, which received only as much food per gram of body weight as was consumed by the vitamin A deficient animal. The positive

controls received food *ad libitum*. After the appearance of conjunctivitis, the calorie and positive controls were given four drops (120 mg.) of cod liver oil daily throughout the entire experiment.

Some of the mothers of the experimental animals were bred while they were being fed on stock diet no. 2 (Sure, '28). The formula for this diet is, ground whole wheat 2640 gm., whole milk powder 1308 gm. and 52 gm. of NaCl. The nurslings were fed by the mothers on this diet, and when needed for experiments were taken directly from stock diet no. 2 and placed on the vitamin A deficient diet no. 1034 (Sure, et al, '33), which consisted of 20 per cent hot alcohol extracted casein, 10 per cent Northwestern yeast, 4 per cent salts no. 185 (McCollum and Simmonds, '17), and 66 per cent dextrin. The mixture was irradiated with a Cooper Hewitt ultraviolet lamp for 30 minutes.

The mothers of the remaining triads were nourished on stock diet no. 1 (Sure, '26) which consisted of 27 per cent whole wheat, 26 per cent rolled oats, 25 per cent yellow corn meal, 15 per cent linseed oil meal, 5 per cent commercial casein, 1 per cent cod liver oil, 0.5 per cent NaCl, and 0.5 per cent  $\text{CaCO}_3$ , with a liberal supply of whole milk. The vitamin A deficient animals of the triads fed on stock diet no. 1 were more resistant to the onset of ophthalmia and lived longer than those whose mothers were fed on stock diet no. 2 (Sure, '28) during gestation and lactation. They evidently had a storage of food constituents, including vitamins, which enabled them to combat a vitamin A deficient diet longer than those fed on stock diet no. 2.

In this study ten triads, or thirty animals, were used, fifteen for the determination of fatty acids and lecithin, and fifteen for cholesterol. Twenty-four of the thirty animals were females. Although the appearance of ophthalmia was taken as the definite indication of vitamin A deficiency, evidence of the pathological condition was first noted with the continuous occurrence of cornified cells in the vaginal smears of the females. This phenomenon appeared from 7 to 15 days be-

fore the symptoms of ophthalmia could be detected. Evans ('28) made a very comprehensive study of the keratinization of uterine epithelium and found the occurrence sufficiently constant to be used as a means of identification of vitamin A deficiency. Aberle ('33) also states that cornification of cells invariably precedes other symptoms of vitamin A avitaminosis.

As soon as the animals were placed on a vitamin A deficient diet,  $\frac{1}{2}$  ml. of peripheral blood was taken once per week from each animal of the fatty acid-lecithin lot, and a similar amount of blood from those from which cholesterol was to be obtained. The animals were always fasted 16 hours before bleeding.

The fatty acids were estimated according to the method of Smith and Kik ('33). The inorganic phosphorus from whole blood from which lecithin was computed, was determined by the Fiske and Subbarow method ('25) modified by F. C. Koch ('34), cholesterol was estimated colorimetrically by use of the Liebermann-Burchard reaction. A complete description of the process is given by Bloor, Pelkan and Allen ('22).

Until the calorie and positive control animals received cod liver oil the dietary regime was constant for all.

As conjunctivitis was taken as the definite symptom of a pathological condition, somewhat similar in all the animals, the amount of lipids determined from the blood at this particular stage in the disease was used as a basis from which to calculate the means when all the animals were fed on a diet deficient in vitamin A.

## RESULTS

Table 1 gives the mean number of milligrams of fatty acids, lecithin and cholesterol in 100 ml. of the blood of the three groups combined, before the administration of cod liver oil to the controls.

After cod liver oil was given to the control animals, the purpose of the triad arrangement becomes more significant.

In the tables the vitamin A deficient animals will be designated P, the calorie controls as R, and the positive controls as C.

TABLE 1

*Mean amounts of fatty acids, cholesterol and lecithin in 100 ml. blood previous to the administration of cod liver oil. Seven blood analyses were made for fatty acids and lecithin and six analyses for cholesterol.*

*Vitamin A deficient animals*

NUMBER OF ANIMALS	FATTY ACIDS		NUMBER OF ANIMALS	CHOLESTEROL		NUMBER OF ANIMALS	LECITHIN	
	M	PE		M	PE		M	PE
	<i>mg.</i>			<i>mg.</i>			<i>mg.</i>	
3	306.3 ± 3.69					3	285.9 ± 2.03	
6	334.4 ± 11.60		3	88.3 ± 2.94		6	306.7 ± 6.74	
6	318.7 ± 6.73		3	88.1 ± 6.03		6	306.9 ± 5.52	
12	297.8 ± 4.45		3	126.8 ± 3.37		12	308.9 ± 4.44	
12	292.1 ± 5.18		8	100.1 ± 5.83		12	289.3 ± 6.73	
12	323.8 ± 7.62		11	114.5 ± 3.24		12	289.0 ± 5.48	
12	306.0 ± 5.13		12	126.5 ± 3.72		12	283.0 ± 3.35	

Cod liver oil given at this point

M, mean. PE, probable error.

Table 2 gives the mean number of milligrams of fatty acids, cholesterol and lecithin per 100 ml. of blood, at each bleeding, for the groups P, R, C, after the two controls have received therapeutic treatment.

Fatty acids and lecithin were determined from the same blood sample. When cholesterol was extracted first, then fatty acids and finally the lecithin from the same blood sample, fatty acids and lecithin were invariably less than when extractions were made from duplicate samples previous to the extraction of cholesterol. For this reason a different set of animals was used for cholesterol determinations.

TABLE 2

*The mean amounts of fatty acids, cholesterol and lecithin in 100 ml. blood for the individual bleedings of each group in the triads after the administration of cod liver oil to the controls*

NUMBER OF ANIMALS	P		R		C	
	M	PE	M	PE	M	PE
Fatty acids						
	mg.		mg.		mg.	
1	248.4 ± 17.28		266.1 ± 17.28		248.4 ± 17.28	
2	330.4 ± 12.21		297.0 ± 12.21		297.0 ± 12.21	
3	328.0 ± 9.98		288.9 ± 9.98		294.1 ± 9.98	
5	323.6 ± 7.73		299.1 ± 7.73		270.9 ± 7.73	
5	279.1 ± 7.73		318.1 ± 7.73		273.9 ± 7.73	
Cholesterol						
3	124.1 ± 3.52		116.1 ± 3.52		108.9 ± 3.52	
4	119.2 ± 2.49		105.1 ± 2.49		107.2 ± 2.49	
4	123.1 ± 2.49		115.8 ± 2.49		111.4 ± 2.23	
5	120.9 ± 2.23		118.7 ± 2.23		107.9 ± 2.23	
5	122.5 ± 2.23		119.8 ± 2.23		104.6 ± 2.23	
5	104.3 ± 2.23		121.3 ± 2.23		112.1 ± 2.23	
Lecithin						
1	285.2 ± 11.16		280.7 ± 11.16		254.0 ± 11.16	
2	299.8 ± 7.94		292.2 ± 7.94		282.0 ± 7.94	
3	324.4 ± 6.48		280.1 ± 6.48		276.2 ± 6.48	
5	295.1 ± 5.02		280.1 ± 5.02		272.3 ± 5.02	
5	325.2 ± 5.02		277.4 ± 5.02		267.0 ± 5.02	

P, vitamin deficient animal. M, mean. R, calorie control. PE, probable error. C, positive control.

Table 3 gives a comparison between groups at each bleeding, after the administration of cod liver oil to the controls. It shows the variations in the amounts of the three blood lipids for the groups P, R, and C.

The differences in fatty acids between P and C gradually increase until the fourth bleeding when the excess becomes highly significant, P being the greater. At the last bleeding the pathological animals are in a moribund condition and the blood fatty acids are similar in amount to those of the positive controls.

TABLE 8

*Comparison between groups at each bleeding after administration of cod liver oil to the controls*

BLOOD SAMPLE	FATTY ACIDS			CHOLESTEROL			LECITHIN		
	Group	Mean amount	$\frac{M_D}{PE_{MD}}$	Group	Mean amount	$\frac{M_D}{PE_{MD}}$	Group	Mean amount	$\frac{M_D}{PE_{MD}}$
1st	R	266.1	1.00	P	124.1	1.29	P	285.2	0.40
	P	248.4		R	116.1		R	280.7	
	R	266.1	1.00	P	124.1	2.46	P	285.2	2.79
	C	248.4		C	108.9		C	254.0	
	P	248.4	0.00	R	116.1	1.16	R	280.7	2.39
	C	248.4		C	108.9		C	254.0	
2nd	P	330.4	1.93	P	119.2	2.63	P	299.8	0.68
	R	297.0		R	105.1		R	292.2	
	P	330.4	1.93	P	119.2	2.24	P	299.8	1.59
	C	297.0		C	107.2		C	282.0	
	C	297.0	0.00	C	107.2	0.37	R	292.2	0.91
	R	297.0		R	105.1		C	282.0	
3rd	P	328.0	2.77	P	123.1	1.30	P	324.4	4.86
	R	288.9		R	115.8		R	280.1	
	P	328.0	2.40	P	123.1	1.40	P	324.4	5.29
	C	294.1		C	111.4		C	276.2	
	C	288.9	0.37	R	115.8	0.57	R	280.1	0.43
	R	294.1		C	111.4		C	276.2	
4th	P	323.6	2.26	P	120.9	0.40	P	295.1	2.12
	R	299.1		R	118.7		R	280.1	
	P	323.6	4.83	P	120.9	2.40	P	295.1	3.23
	C	270.9		C	107.9		C	272.3	
	R	299.1	2.59	R	118.7	1.99	R	280.1	1.00
	C	270.9		C	107.9		C	272.3	
5th	R	318.1	3.58	P	122.5	0.50	P	325.2	6.77
	P	279.1		R	119.8		R	277.4	
	R	318.1	4.05	P	122.5	3.30	P	325.2	8.24
	C	273.9		C	104.6		C	267.0	
	P	279.1	0.48	R	119.8	2.80	R	277.4	1.47
	C	273.9		C	104.6		C	267.0	
6th				R	121.3	3.30			
				P	103.4				
				C	112.1	1.42			
				P	103.4				
				R	121.3	1.69			
				C	112.1				

M, mean.  $M_D$ , mean difference.  $PE_{MD}$ , probable error of mean difference.  $\frac{MD}{PE_{MD}}$  significant ratio. 2.5 to 2.8 approaching significance: 2.8 to 3.0 significance: above 3.0 is high significance.



P significantly exceeds R in fatty acids at the third bleeding. At the fourth the excess is small. At the last bleeding the excess of R over P is highly significant. The excess of R over C approaches significance in the bleeding before the last and becomes highly significant at the last.

A similar comparison between groups at each bleeding to determine differences in cholesterol, shows that P exceeds C significantly at the fifth bleeding, which is the one before the last. In the first four bleedings there is no uniform gradation of differences. At the last bleeding, C is slightly in excess of P. The excess of P over R for cholesterol approaches significance at the second bleeding and after that diminishes to insignificance. At the last bleeding the difference is inverted and R significantly exceeds P. The cholesterol content of the blood of the R group is slightly in excess of C from the third bleeding. At the last bleeding, P is the least of the three groups.

Because the concentration of the fatty acids and cholesterol of the blood of the vitamin A deficient animals became significantly less when the animals were in a moribund condition and the lecithin remained higher than that of either of the controls until the end of the experiment, fatty acids and cholesterol were placed consecutively in the tables, notwithstanding the fact that they were obtained from different blood samples.

The comparison between groups at each bleeding for lecithin, after the controls had received therapeutic treatment, showed that P exceeds R significantly at the third and fifth bleedings, but the differences are not significant in the other three cases. P exceeds C significantly in all the bleedings except the second. In contrast to the results obtained for fatty acids and cholesterol, the lecithin content of the blood of the vitamin A deficient animals at the last bleeding shows differences in excess of the two controls which are highly significant.

These results are very similar to those obtained by Asada ('23 a, b, c). He states that Susuki explained the decrease

in fat oxidation in avitaminosis as due to the lack of vitamin A, which oxidizes fat. When there is a decrease in fat oxidation the protoplasmic cells do not absorb the blood fat with the same speed, and the fat remains in the blood at a higher level. Later in avitaminosis, the blood fat decreases perhaps without reaching the normal value, due to the fact that the fat depots are exhausted and do not give any more to the blood. Asada, Susuki (quoted by Asada) and Collazo and Bosch ('23) showed that with avitaminotic animals, blood fat and cholesterol increased. The amount of increase depended upon the phase of the disease and especially upon the intensity with which the fat depots were emptied.

Table 1, on page 679, gives the mean amounts of fatty acids, cholesterol and lecithin at each bleeding for all the animals before the administration of cod liver oil to the controls. Table 2, on page 680, records the mean amounts of the three lipid constituents, at each bleeding, for the groups, P, R, and C after the controls received therapeutic treatment.

Table 3, which is the result of a more detailed statistical analysis, shows that the blood fatty acids and cholesterol of the vitamin A deficient animals exceed those of the control groups until the last bleeding. Then an inversion occurs. During the last week of the experiment the vitamin A deficient animals were very ill, consumed little food, and lost weight rapidly. It is reasonable that at this stage of the disease, the fat depots would be depleted of their supply, and the amount of fat furnished to the blood relatively decreased.

Wendt ('28) worked with starving animals and concluded from experimental results that during the first few days of starvation much lipid material came from the transformed fatty tissue and was liberated into the blood stream.

In this experiment the calorie controls received the same amount of food per gram of body weight as was consumed by the vitamin A deficient animal of the same triad. During the last week of the experiment the calorie controls were inadequately nourished and, being healthy animals, had to depend upon endogenous reserves in order to carry on normal

body processes. The amounts of fatty acids and cholesterol in the blood of the animals increased on account of the transportation of these substances from the fat depots to the places of combustion.

The amount of lecithin in the blood of the vitamin A deficient animals remained in excess of the two controls until the end of the experiment. Degkwitz ('31) has made a recent review of the literature on lecithin/cholesterol balance. He finds that results show an essential antagonism between these two substances as regards cellular physiology.

Henrickson ('29) studied "General cell degeneration as a result of vitamin A deficiency" and found that rats in advanced stages of avitaminosis A showed general cell degeneration in all the organs. No postmortem examinations of tissues were made in this study and, therefore, no comparisons are justified except in the cases of continuous appearance of cornified cells in the vaginal smears and xerophthalmia which are cited in the order of their occurrence.

A comparison between groups at each bleeding after the administration of cod liver oil to the controls showed significant difference as avitaminosis advanced (table 3). In order to check these results the differences between groups, triads, and individual bleedings were obtained by 'analysis of variance' (Fisher, '30).

In determining the significance of various contributors to variance, interpretations were made by the use of 'F' (Snedecor, '34). This analysis shows a highly significant contribution of variance in the groups (P, R, and C) for fatty acids, cholesterol and lecithin. The contribution of variance due to bleedings was not significant for lecithin and cholesterol, but did show significance for fatty acids. The variance due to the triads was highly significant for lecithin and cholesterol. The triads, the mothers of which were fed on stock diet no. 1, had a higher lecithin and cholesterol concentration in the blood than those whose subsistence could be traced back to stock diet no. 2.

The precipitous fall in fatty acids and cholesterol when the vitamin A deficient animals were in a moribund condition was characteristic of this avitaminosis and independent of dietary regime.

The contribution of variance due to sex was not significant for any of the three blood lipids studied in this experiment.

#### SUMMARY

1. A diet deficient in vitamin A produced a continuous occurrence of cornified cells in the vaginal smears of the female albino rats, from 7 to 15 days before the appearance of ophthalmia.

2. After the administration of cod liver oil to calorie and positive controls the fatty acid and cholesterol content of the blood of the vitamin A deficient animals exceeded that of the two controls. At the bleeding before the last, the fatty acids and cholesterol of the vitamin A deficient animals significantly exceeded the calorie and positive controls. An inversion was evident at the last bleeding. The calorie controls then showed a significant excess of fatty acids and cholesterol over the vitamin A deficient animals.

3. From the third to the last bleedings, inclusive, the lecithin content of the blood of the vitamin A deficient animals was significantly higher than that of either of the controls, with the exception of the fourth bleeding, when the difference between vitamin A deficient animals and calorie controls was only slightly significant.

4. In the comparison between groups at each bleeding after the administration of cod liver oil, analysis of variance was applied to test for significant differences between groups, triads and individual bleedings. This analysis proved a highly significant difference between groups for fatty acids, cholesterol and lecithin. The differences between the individual bleedings were found to be significant only for the fatty acids.

5. The differences between the triads were significant for lecithin and cholesterol. The reason for these variations was traced back to the nutritional background of the animal.

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#### LITERATURE CITED

- ABERLE, S. B. D. 1933 Continual cornified vaginal cells as an index of avitaminosis in rats. *J. Nutrition*, vol. 5, p. 1.
- ASADA, KAZUO 1923 a Fat metabolism in avitaminosis. Part I. Total fat and cholesterol content of the body in normal and avitaminotic nutrition. *Biochem. Zeitschr.*, Bd. 141, S. 166.
- 1923 b Fat metabolism in avitaminosis. Part II. The content of total fat, neutral fat, cholesterol and cholesterol ester in blood of normal, fasting, avitaminotic, and phosphor poisoned rats. *Biochem. Zeitschr.*, Bd. 142, S. 144.
- 1923 c Fat metabolism in avitaminosis. Part III. The fat and cholesterol content of the liver after phosphorus poisoning—in normal, fasting, and avitaminotic rats. *Biochem. Zeitschr.*, Bd. 142, S. 165.
- BLOOR, W. R., K. F. PELKAN AND D. M. ALLEN 1922 Determination of fatty acids (and cholesterol) in small amounts of blood plasma. *J. Biol. Chem.*, vol. 52, p. 191.
- COLLAZO, J. A., AND GOMEZ BOSCH 1923 Blood fat content in avitaminosis. *Biochem. Zeitschr.*, Bd. 141, S. 370.
- DEGKWITZ, R. 1931 Lecithin/cholesterol balance. *Ergebnisse physiol. Chem.*, Bd. 32, S. 821. Cited from *Ann. Rev. of Biochemistry*, vol. 2, p. 160, Stanford University Press, 1933.
- EVANS, H. M. 1928 Effects of inadequate vitamin A on sexual physiology of the female. *J. Biol. Chem.*, vol. 77, p. 651.
- FISHER, R. D. 1930 Statistical methods for research workers. p. 202. Oliver & Boyd, London.
- FISKE, C. H., AND Y. SUBBAROW 1925 The colorimetric determination of phosphorus. *J. Biol. Chem.*, vol. 66, p. 375.
- FUKUNDA, TAMOTSU 1930 Change of the fat content of blood in animals fed with vitamin-poor food. *Med. News, Japan*, no. 1243, pp. 20-35. Copied from *Chem. Abstr.*, p. 1146, 1930.

- HENRICKSON, P. 1929 Cell degeneration as a result of vitamin A deficiency: cod liver oil in large doses. *Acta, Paediat.*, vol. 8, p. 397. Copied from *Am. J. Dis. Children*, vol. 39, p. 192, January, 1930.
- KOCH, F. C. 1934 Practical methods in biochemistry, pp. 150-152. William Wood & Company, Baltimore.
- MCCOLLUM, E. V., AND N. SIMMONDS 1917 Pellagra producing diets. *J. Biol. Chem.*, vol. 32, pp. 347-355.
- SMITH, M. E., AND M. C. KIK 1933 A micromethod for the determination of fatty acids from small amounts of whole blood. *J. Biol. Chem.*, vol. 103, p. 391.
- SNEDECOR, G. W. 1934 Calculation and interpretation of analysis of variance. Collegiate Press, Inc., Ames, Iowa, p. 88.
- SURE, B. 1926 The existence of a lactation promoting factor in the unsaponifiable matter from wheat oil. *J. Biol. Chem.*, vol. 69, p. 53.
- 1928 Dietary requirements for fertility and lactation. XIII. Storage of fat-soluble vitamins for lactation, with some observations on the cod liver oil requirements of nursing young. *J. Biol. Chem.*, vol. 76, p. 660.
- SURE, B., M. C. KIK, DOROTHY J. WALKER AND MARGARET E. SMITH 1933 Biochemistry and pathology of avitaminosis. I. Hematopoietic function in deficiency diseases. *Ark. Expt. Sta. Bul.* 286, p. 4.
- WENDT, H. 1928 Lipoid metabolism studies on starving animals. *Klin. Wochenschr.*, Bd. 7, S. 218-225.



# THE RELATION BETWEEN THE ANTIRACHITIC FACTOR AND THE WEIGHT OF THE GALL BLADDER AND CONTENTS OF THE CHICKEN<sup>1</sup>

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Brief reference (Russell and Chichester, '31) has already been made regarding the observation that the average weight of gall bladders and contents of chicks receiving a ration deficient in the antirachitic factor is greater than that of normal chicks. Loeffler ('28) has called attention to the enlargement of the gall bladder of the mouse when bile acids were fed, but no mention was made of the presence or absence of an antirachitic substance in the diet. This paper is a presentation of further data concerning the observation previously reported.

## *Experiment 1*

*Procedure.* The four groups of White Wyandotte chicks used in this study were fed a basal ration, with or without a supplement, as indicated in column 1 of table 1, which consisted of 47 per cent ground yellow corn, 20 per cent wheat middlings, 15 per cent wheat bran, 5 per cent dried buttermilk, 9 per cent meat scrap (55 per cent protein), 3 per cent ground oyster shell and 1 per cent sodium chloride. Cod liver oil was administered by capsule, five or six times per week, in an amount based upon the weekly consumption of

<sup>1</sup>Journal series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.



a ration containing 1 per cent of the oil (Card and Kirkpatrick, '18), existing data on food consumption by another heavy breed, Rhode Island Reds, being used for the calculation. Irradiated ergosterol in corn oil and corn oil alone were administered in the same manner as the cod liver oil and in the same volume. As indicated in table 1, the number of rat units of the antirachitic factor in the form of irradiated ergosterol was six times that fed as cod liver oil. The chicks

TABLE 1  
*Summary of data obtained at 7.5 weeks of age*

GROUP NUMBER AND TREATMENT	NUMBER OF BIRDS	AVERAGE BODY WEIGHT AT 7.5 WEEKS OF AGE	AVERAGE WEIGHT OF GALL BLADDERS AND CONTENTS	MAXIMUM AND MINIMUM WEIGHTS OF GALL BLADDERS AND CONTENTS	COEFFICIENT OF VARIATION	BONE ASH
1 Cod liver oil 1 per cent level	36	gm. 557	mg. 294	mg. 564 70	per cent 39	per cent 47.93
2 Irradiated ergosterol in corn oil. Equivalent to 6 per cent cod liver oil	31	474	543	1027 160	46	44.45
3 Corn oil. Same volume as groups 1 and 2	12	371	1195	3827 421	74	40.07
4 Basal	29	337	699	2270 128	71	39.69

were killed by decapitation, and the gall bladders and contents dissected out and weighed in a weighing bottle. Bone ash was determined by a method previously described (Russell and Massengale, '28).

*Results and discussion.* Upon the basis of bone ash percentage and body weight, group 1 (table 1) is classed as normal for the breed at the age of killing. The coefficients of variation indicate that there is considerable variation of values within a group, but the average weight of the gall bladders and contents of group 1, the normal group, is

significantly lower than those of the irradiated ergosterol, corn oil, and basal groups. The gall bladders of the deficient birds were turgid with bile in practically every case, whereas this condition occurred rarely in the protected groups. When those of the deficient groups were not filled with bile, they had a stretched, flaccid appearance. In contrast, those of the cod liver oil groups were usually in a contracted state. The bone ash percentage of the irradiated ergosterol group lies between that of the normal and those of the groups deprived of the factor and indicates incomplete protection against leg weakness. It is of interest that the average weight of the gall bladders of the irradiated ergosterol group also lies between the values for the two groups just mentioned. As stated above, the antirachitic potency of the irradiated ergosterol is six times that of cod liver oil on the basis of a rat assay. These facts suggest that gall bladder size in the chicken is associated with the effectiveness of the factor in promoting bone formation, and not with the number of rat units consumed.

The basal group did not receive an oil supplement, but the other groups were given an amount of oil per week, corn oil or cod liver oil, which increased from 0.5 cc. during the first week to 3.6 cc. during the last week of the experiment. Although the average weight for the corn oil group is higher than the others, the difference between the average weights of gall bladders of this and the basal group does not have much statistical significance, because of the high coefficients of variation and probable errors involved, and the small population of the corn oil group. The observation suggests further study of the effect of fat in the ration, with and without the antirachitic factor, on the size of the gall bladder.

### *Experiment 2*

Ivy and Oldberg ('28) have shown that the injection of hydrochloric acid into the duodenum of the dog under barbital anesthesia caused the gall bladder to contract. Hence the question was raised whether there was a difference in H-ion

concentration of the duodenal contents of birds with and without the antirachitic factor, which might account for the greater bile content of the bladders of birds deprived of the factor. Also it was of interest to determine whether there were differences in total solids, ash, and calcium contents of the biles from the two groups.

*Procedure.* Duodenal contents were available from two groups of chicks at 8 weeks and bile at 5, 8, and 11 weeks of age. Both groups were fed the basal ration described under experiment 1, but group 1 was protected against leg weakness by the incorporation of 1 per cent cod liver oil in the ration, whereas group 2 was deprived of the antirachitic factor. Immediately after killing, the duodenal loop was removed and the H-ion concentration of the contents determined by means of the quinhydrone electrode (Cullen and Billman, '25). The bile from each group was pooled and total solids were determined at 98° to 100°, the ash by destruction of organic matter in an electric muffle, and calcium of the ash volumetrically (Official Methods, '30).

#### RESULTS AND DISCUSSION

The group which received cod liver oil in the ration was adequately protected against leg weakness, but the basal group had developed severe leg weakness at 8 weeks of age. Although gall bladder weights were not obtained, as was done previously, the average volume of bile from the basal group, 0.41 cc., was more than twice that, 0.16 cc., from the protected group. The gall bladders of the deficient birds were markedly enlarged and turgid with bile as compared with the cod liver oil group, as was also observed in experiment 1.

The average pH of the duodenal contents of the basal group was 6.36 and of the protected group 6.46 at 8 weeks of age. This close agreement shows that H-ion concentration is not a factor which controls the amount of bile in the bladder under these nutritional conditions.

The total solids of the bile of birds which had received the antirachitic factor was practically constant throughout the

experiment, table 2, whereas the value for the basal group increased with age. The percentage of ash was found to fluctuate in the protected group, but a tendency to increase with age is again apparent in the deficient birds. Calcium expressed as mg. per 100 gm. of bile was 25 to 35 per cent lower in the basal as compared with the protected group, but the average volume of bile of the basal group at 8 weeks of age was of the order of twice that of the normal group. Hence

TABLE 2

*Total solids, ash, and calcium content of pooled gall bladder bile*

GROUP NO., SUPPLEMENT AND AGE OF KILLING	NUMBER OF BIRDS	TOTAL SOLIDS	ASH	CALCIUM
		<i>per cent</i>	<i>per cent</i>	<i>mg./100 gm. of bile</i>
1 1 per cent cod liver oil. Protective				
5 weeks	15	19.45	2.02	63.6
8 weeks	12	19.55	2.13	61.1
11 weeks	12	19.30	1.98	61.6
2 Basal				
5 weeks	15	18.45	1.91	46.0
8 weeks	12	20.50	2.20	39.6
11 weeks	12	21.65	2.30	45.3

although the concentration of calcium was lower in the bladder bile of the basal group, the total calcium in the bile of these bladders is probably greater than that in the bladders of the protected group. Whether more calcium goes into the intestine from the deficient bird than from the normal is not known, but would depend upon the frequency of the emptying of the bladder. If the enlarged gall bladders are indicative of greater excretion of bile into the intestine, the bile may be an important excretory route for calcium in the leg weakness condition.

## SUMMARY

1. The average weight of the gall bladders and contents of a group of chicks deprived of the antirachitic factor was more than twice that of a group provided with an adequate amount of the factor.

2. When a group of chicks was only partially protected against leg weakness, as indicated by the percentage bone ash, the average weight of the gall bladders was intermediate between the values observed for a normal group and for one deprived of the factor.

3. The average H-ion concentration of the duodenal contents was essentially the same whether the groups had an adequate amount or were deprived of the antirachitic factor, and is apparently not related to the retention of bile by the gall bladder.

4. Total solids, ash and calcium were determined in the bile at 8 weeks of age.

## LITERATURE CITED

- ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1930 Official and tentative methods of analysis. Washington, 3rd ed., p. 104.
- CARD, L. E., AND W. F. KIRKPATRICK 1918 Rearing chickens. I. Normal rate of growth in White Leghorns and Rhode Island Reds. Conn. Agric. Exp. Sta. (Storrs), Bull. 96.
- CULLEN, G. E., AND E. BILLMAN 1925 The use of the quinhydrone electrode for hydron concentration determination of serum. J. Biol. Chem., vol. 64, p. 727.
- IVY, A. C., AND E. OLDBERG 1928 A hormone mechanism for gall-bladder contraction and evacuation. Am. J. Physiol., vol. 86, p. 599.
- LOEFFLER, K. 1928 The relation of bile acids to alimentary cholesterol. Zeit. physiol. Chem., Bd. 178, S. 186.
- RUSSELL, W. C., AND D. F. CHICHESTER 1931 The gall bladders of chicks in a vitamin D deficient condition. Science, vol. 74, p. 437.
- RUSSELL, W. C., AND O. N. MASSENGALE 1928 The effect on bone formation of winter sunlight transmitted by a glass substitute. Poultry Sci., vol. 7, p. 85.

# THE BASAL METABOLISM OF EUROPEAN WOMEN IN SOUTH INDIA AND THE EFFECT OF CHANGE OF CLIMATE ON EUROPEAN AND SOUTH INDIAN WOMEN

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## FOUR FIGURES

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In presenting their data on the metabolism of south Indian women, Mason and Benedict ('31) suggested possible causes other than race which might be found to be factors in the low metabolism of this group. Among these causes was the factor of climate. If the tropical climate as such affects human metabolism that effect should certainly be apparent in the conditions prevailing in Madras. The city of Madras, lying on the southeast coast of India, 13° 4" north of the equator at an altitude of 22 feet, has a mean annual temperature of 82.2° F., a relative humidity of 72 per cent, and a barometric pressure of 758.0 mm. The seasonal variation is slight, the highest monthly maximum temperature, 98.2° F., occurring in June, the lowest, 83.7°, in December. The lowest monthly minimum, 67.5°, occurs in January, the highest, 80.8°, in May. The monthly variations in humidity are from 62 per cent in June to 79 per cent in the rainy season in November. The barometer shows pressure variations of from 754.3 mm. in June to 761.8 mm. in January.

<sup>1</sup>This study was made possible by the loan of metabolism apparatus and by financial assistance from the Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.

Apart from a study of the races which are native to Madras, in whom the climatic factor may be obscured by other factors, such as activity or dietary habits or race itself, the effect of the Madras climate may be studied either by finding whether the members of white races residing in Madras show a different metabolism from those of the same race in temperate climates, or, better, by studying the effect of change of climate on the same individuals, both European and Indian, as they move their residence from one country to another. Both methods have been used in the experiments reported here.

#### SUBJECTS

Thirty-four European women, residents of Madras or its immediate environs, were measured. Table 1 shows their ages, race and physical measurements together with data concerning their association with the tropics. There was a wide range of age and considerable difference in configuration. The total length of residence in the tropics varied from 2½ months to 31 years, but the stays of longer duration had been interrupted by periodic furloughs. Of these thirty-four European women measured in Madras nine were studied more extensively for the effect of change of climate. In addition, measurements were secured on three of their Indian colleagues who went abroad to study.<sup>2</sup>

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I wish also to thank my two colleagues who made the measurements on me in Madras, Miss Beatrice S. Cosmey and Dr. Mariam P. Oommen.

TABLE 1

*Physical characteristics and other data concerning European women in Madras*

NUMBER AND SUBJECT	RACE	AGE	TOTAL TIME IN INDIA			TIME SINCE LAST ARRIVAL IN INDIA		WEIGHT	HEIGHT	SITTING HEIGHT	PELIDISI <sup>1</sup>
			Years	Years	Months	Years	Months				
1 W.D.	English	20	8			1		56.4	159	84.5	97
2 S.H.	Danish	21	9	6		3		56.2	160	83.0	99
3 G.E.S.	American	23		4			4	54.0	167	88.5	92
4 G.G.	Scotch	24	5	5			5	59.6	165	88.0	96
5 M.S.	English	25	1	1		1	1	50.3	175	91.5	86
6 M.H.	English	25		2½			2½	50.8	165	88.5	90
7 E.L.C.	Canadian	26		7			7	45.8	154	86.0	90
8 K.S.	English	27	1			1		45.8	161	85.0	91
9 B.S.C.	American	28	1	8		1	8	59.9	157	85.0	99
10 D.H.	English	29	3			3		55.4	170	91.0	90
11 M.R.B.	English	29	1	8		1	8	55.1	159	83.0	99
12 A.M.P.	Scotch	30		5½			5½	47.6	156	83.0	94
13 H.H.	Scotch	31		5½			5½	65.0	162	86.5	100
14 E.S.	English	31	5			5		49.9	162	83.0	96
15 D.E.H.	English	32	6			1	6	58.1	168	88.0	95
16 M.P.	English	32	5			5		50.3	170	89.5	88
17 E.D.M.	American	32	9			1	9	47.7	169	86.0	91
18 E.P.	Danish	32	1	9		1	9	54.3	175	88.5	92
19 M.D.B.	American	36	10			5		52.2	160	85.5	94
20 S.C.F.	American	37	19			1	8	50.3	162	88.5	89
21 O.E.J.	American	38	8			2	2	44.9	159	80.0	96
22 O.M.S.	American	38	10				9	50.2	159	84.0	95
23 E.J.	English	39	10			1	1	50.5	158	84.0	95
24 A.M.V.	English	40	13			1	6	68.0	170	89.0	99
25 K.N.B.	English	41	16			2	2	57.4	167	87.5	94
26 E.M.C.	American	41	11				11	52.4	165	85.5	94
27 I.McN.	Scotch	42	11				11	71.1	169	89.5	99
28 M.L.D.	English	44	18			1	6	53.5	160	84.5	96
29 A.G.G.	Scotch	45	1			1		48.5	161	85.0	92
30 J.E.B.	American	46	12				4	53.1	156	84.5	95
31 G.E.C.	American	47	31			2	7	70.3	168	87.0	102
32 A.V.D.	American	50	23				6	58.9	166	85.0	99
33 A.G.S.	American	54	1	8		1	8	56.7	154	79.5	103
34 E.McD.	English	54	11			1	9	57.4	164	84.0	99
Average—34 Europeans		35						54.6	163	85.9	95
Average—54 Indians <sup>2</sup>		21						45.0	154	78.5	97.5

<sup>1</sup> Calculated from formula of von Pirquet.<sup>2</sup> Reported by Mason and Benedict ('31).



## PROCEDURE

For the measurements in Madras the subjects came to the laboratory on intermenstrual days at 7.00 A.M. and rested for at least half an hour before the beginning of the experiment. The usual hour for the preceding meal was the same as in the Indian series, 7.30 P.M. The point raised by Radsma ('31) that a half-hour rest period may not be long enough for tropical residents to achieve a state of repose has been discussed in a paper on the effect of sleep on metabolism (Mason and Benedict, '34). Mouth temperatures were taken during the rest period with the thermometer in position for at least 5 minutes. Reclining blood pressures were taken with a mercury manometer at the end of the experiment.

The apparatus used for measuring the oxygen consumption was the Benedict spirometer type, with graphic record of respiration. In all experiments through July, 1930, a mouthpiece was used; after that time the mouthpiece was replaced by the Benedict helmet (Benedict, '30).

The measurements in temperate climates were all made in well-established laboratories and clinics and the experimenters followed the customary procedures necessary to secure basal conditions.

## RESULTS

*A. Physiological measurements on European women in Madras and comparison with Indian women*

In table 2 are shown the averages of measurements for each of the thirty-four women studied in Madras. All, except two, were measured on at least two consecutive or nearly consecutive days and those two had had trial experiments previous to the day recorded. The figures given represent, on an average, for pulse rate eighteen measurements, for respiration rate twelve measurements, blood pressure four measurements, and oxygen consumption six 10-minute periods of measurement. The recorded vital capacity was the highest of three readings. For comparison with these data, the corresponding data on Indian women reported in 1931 are included.

TABLE 2  
Summary of measurements on European women in Madras

NUMBER	MOUTH TEMPERATURE ° F.	PULSE RATE	BLOOD PRESSURE			RESPIRATION RATE	VITAL CAPACITY	O <sub>2</sub> CC./MIN.	HEAT PRODUCTION		PER CENT DEVIATION		
			Syst.	Diast.	P. Pr.				Per 24 hours	Per m <sup>2</sup> /hr.	Harris Benedict	Dreyer	Abb. DuBois
1	98.8	57	108	62	46	11	2.80	182	Calories 1265	33.6	9.3	-10.5	-11.7
2	98.7	73	109	63	47	14	3.12	185	1286	33.9	7.6	-8.1	-8.4
3	97.9	64	97	60	38	14	4.24	166	1153	30.0	16.1	-15.0	-18.9
4	98.4	49	94	47	47	16	4.05	194	1348	33.8	4.9	-4.8	-8.6
5	98.0	48	101	65	36	13	3.88	167	1160	30.2	13.7	-10.6	-18.4
6	98.6	54	115	50	66	15	3.70	169	1171	31.5	11.9	-10.2	-15.0
7	98.1	58	102	68	34	13	2.80	169	1171	34.6	6.8	-4.9	-6.5
8	98.7	58	115	57	58	12	2.93	156	1094	31.0	14.4	-11.6	-16.4
9	97.9	61	104	66	36	15	2.63	172	1195	31.0	14.0	-14.2	-16.4
10	98.7	71	118	76	42	11	3.72	176	1233	31.3	10.3	-8.3	-15.5
11	98.8	71	108	52	56	22	2.53	185	1283	34.2	4.4	-3.3	-7.6
12	99.0	63	127	77	50	15	4.17	173	1202	34.5	4.5	-2.1	-5.4
13	98.3	75	102	64	38	14	3.61	189	1313	32.4	8.3	-8.2	-11.3
14	98.4	65	112	70	42	19	3.00	168	1164	31.9	9.6	-7.3	-12.7
15	98.8	69	110	83	27	19	3.77	187	1299	32.6	5.3	-3.5	-10.7
16	98.0	57	103	45	58	20	4.03	182	1265	33.5	2.9	+ 0.7	-8.1
17	97.7	60	104	70	34	14	2.78	152	1056	28.6	17.2	-13.3	-21.8
18	98.6	62	107	66	41	17	2.30	187	1299	32.6	3.6	-0.4	-10.6
19	98.6	62	109	68	41	12	2.80	183	1272	34.6	0.8	+ 1.4	-5.2
20	98.4	61	100	59	41	15	3.38	152	1056	29.0	16.4	-14.2	-20.7
21	98.3	70	105	69	36	14	2.60	163	1130	32.9	5.6	-2.4	-9.9
22	98.9	49	105	44	62	16	3.30	176	1233	34.0	2.3	+ 0.2	-6.9
23	98.7	72	98	61	37	21	2.83	163	1133	31.4	9.3	-7.3	-13.8
24	98.4	63	106	74	32	11	3.55	198	1376	32.0	3.9	-2.7	-12.2
25	97.6	74	109	68	41	15	3.60	175	1213	30.8	8.2	-6.4	-14.4
26	99.0	80	121	79	42	13	3.17	187	1299	34.7	+ 2.4	+ 4.9	-3.7
27	98.5	73	116	68	48	19	3.52	208	1442	33.2	0.7	+ 0.4	-7.8
28	97.7	62	107	67	40	10	2.75	166	1150	30.9	9.2	-7.1	-14.1
29	97.9	49	99	57	42	19	2.53	164	1136	31.6	5.8	-3.3	-12.4
30	98.0	54	110	70	40	12	2.60	150	1042	28.8	15.7	-14.9	-20.1
31	98.0	65	105	62	43	17	2.43	174	1206	32.1	14.9	-14.4	-22.1
32	97.5	74	111	80	31	14	3.87	187	1296	32.7	+ 0.4	+ 1.3	-9.2
33	98.6	67	109	71	38	15	3.05	161	1116	30.2	9.3	-9.9	-13.8
34	98.0	63	128	81	47	8	3.05	170	1181	30.4	5.5	-5.4	-13.2
Average 34 Europeans	98.3	63	108	65	43	15	3.21	175	1213	32.0	- 7.9	- 6.3	-12.5
Average 54 Indians	98.4	68	102	64	38	19	2.15 <sup>1</sup>	150	1048	31.2	-16.9	-16.2	-17.2

<sup>1</sup> This figure is based on measurements of 853 south Indian women reported in 1932.

*Mouth temperature.* I have found in the literature very few data on early morning basal temperatures of women in temperate climates for comparison with this series in the tropics. The early morning mouth temperatures of thirteen young women studied by Gustafson and Benedict ('28) at Wellesley College averaged  $97.7^{\circ}$  F. Griffith and his collaborators ('29) found an average mouth temperature under basal conditions of  $97.8^{\circ}$  for three women studied over a long period of time, and the average early morning mouth temperature of five women reported by Harvey and Crockett ('32) was  $97.7^{\circ}$ . Brandt and Benjamin ('33 and unpublished data) found an average basal temperature of  $98.0^{\circ}$  for seven women and Turner (unpublished data)  $98.0^{\circ}$  for eighteen women students. The only exception to these low morning temperatures is a series of six women measured by Carpenter (unpublished data) during a hot spell in Boston in May and June. The average was  $98.3^{\circ}$ . The series in Madras averaged  $98.3^{\circ}$  for the Europeans and  $98.4^{\circ}$  for the Indians. The Madras temperatures early in the morning are therefore a little higher than those of women in temperate climates. It may be noted that eleven of the thirty-four European subjects, or 32 per cent, had temperatures higher than  $98.6^{\circ}$  even at 7.15 A.M. This was true also of thirteen, or 24 per cent, of the fifty-four south Indian women and I believe that these high temperatures should be recognized as within the normal range for women resident in the tropics.

*Pulse rate.* The average reclining pulse rate for the Europeans was 63 beats per minute. This is only half a beat less than the average for thirty-three American women in Benedict's second normal series ('28), but is 6 beats less than the earlier series of Harris and Benedict on 103 women ('19). These two series show approximately the same age range as the Madras series. In the series of twenty-five women 17 to 37 years of age reported by Turner ('27 a) the average reclining pulse was 65. These data therefore indicate that the reclining pulse rate of European women in Madras is as low or slightly lower than that of women in America. The higher

value for Indian women is probably due to their smaller body size rather than to a racial difference.

*Blood pressure.* Like body temperatures, data on reclining blood pressures of women in temperate climates are extremely scarce. The values found for women in Madras are within the range of reclining pressures indicated by Turner ('27 b) for young American women, but the systolic pressure of the Indian group is near the lower limit and in the European group, although twelve were 40 years or older, the systolic level is below the middle of Turner's range for young women. There is a suggestion, then, of a slightly lowered systolic pressure for women in Madras, but the difference may be found to be due to the fact that the group there were in the post-absorptive state. In view of the difference in the ages of the two groups, the small difference in systolic pressure between the Indian and European women is probably not significant.

*Respiration rate.* A comparison of the respiration rate with data obtained in a temperate climate is of questionable value because of possible varying effects on this measurement of the different breathing appliances used with metabolism apparatus. However, since the same apparatus was used with both groups of subjects in Madras, it is justifiable to conclude that there is a probable significance in the difference between the European respiration average of 15 per minute and the Indian average of 19. The more rapid rate in Indian women is probably a compensation for the very shallow breathing that characterizes their respiration records in comparison with the European records.

*Vital capacity.* The vital capacity of the European women is less than 3 per cent lower than the normal prediction of Turner ('30) for American women of the same height. There is, then, no evidence of a climatic effect on this function. The extraordinarily low vital capacity indicated here for the Indian women has been established in a series of measurements on 853 subjects reported in 1932.

*Heat production.* In comparing the heat production of European women in Madras with women in temperate climates and with Indian women, the difficulty is at once apparent that while the metabolism of Indian women is equally low whether compared with the Harris-Benedict, Dryer or Aub-DuBois standards, these same standards vary widely when applied to the European group. In fact, the difference between the deviations from the Dreyer and the Aub-DuBois standards in the European group is greater than the difference between the European and Indian groups by the Aub-DuBois standard. In the European group the deviations from the Aub-DuBois standards diverge more widely from the other two, particularly from the Harris-Benedict, with increasing age, and the interpretation of results is confused by this divergence. If the Harris-Benedict and Dryer standards are used, it may be concluded that the climatic effect on the metabolism of this group as a whole is small, especially if the 5 per cent reduction proposed by Benedict ('28) for women is applied, but between this group and the Indian group there is by the same two standards the significant difference of 9.0 and 10.2 per cent, which remains to be explained by factors other than climate. On the other hand, if the Aub-DuBois standards are applied, the climatic effect is much more marked and the difference between the two racial groups becomes less significant.

*B. The effect of change of climate on European and Indian women*

The confusion of interpretation that is presented by diverging standards is not present in a study of the same individuals as they move from one place to another. Such studies in which the effect of changes of climate on the same individuals has been measured are extremely few. They have been reported by Knipping ('23), Williams and Benedict ('28) and Martin ('30). Knipping measured fourteen European men during a voyage to the tropics. Unfortunately, none of the fourteen were measured on more than one day in their

home ports—a fact which detracts somewhat from the value of the tropical comparison. Following the original measurement, five were measured on 2 days in tropical ports (including Suez and the Red Sea) and nine on 1 day. The results show an average fall in oxygen consumption in the tropics of 2.3 per cent, with variations in different individuals of from + 8.0 to - 9.2 per cent. Although Knipping draws the conclusion that there is an initial rise in the metabolism on going to the tropics with return to normal and subsequent fall after prolonged stay, I think that the questions of the initial rise and the interval at which the fall occurs need further examination.

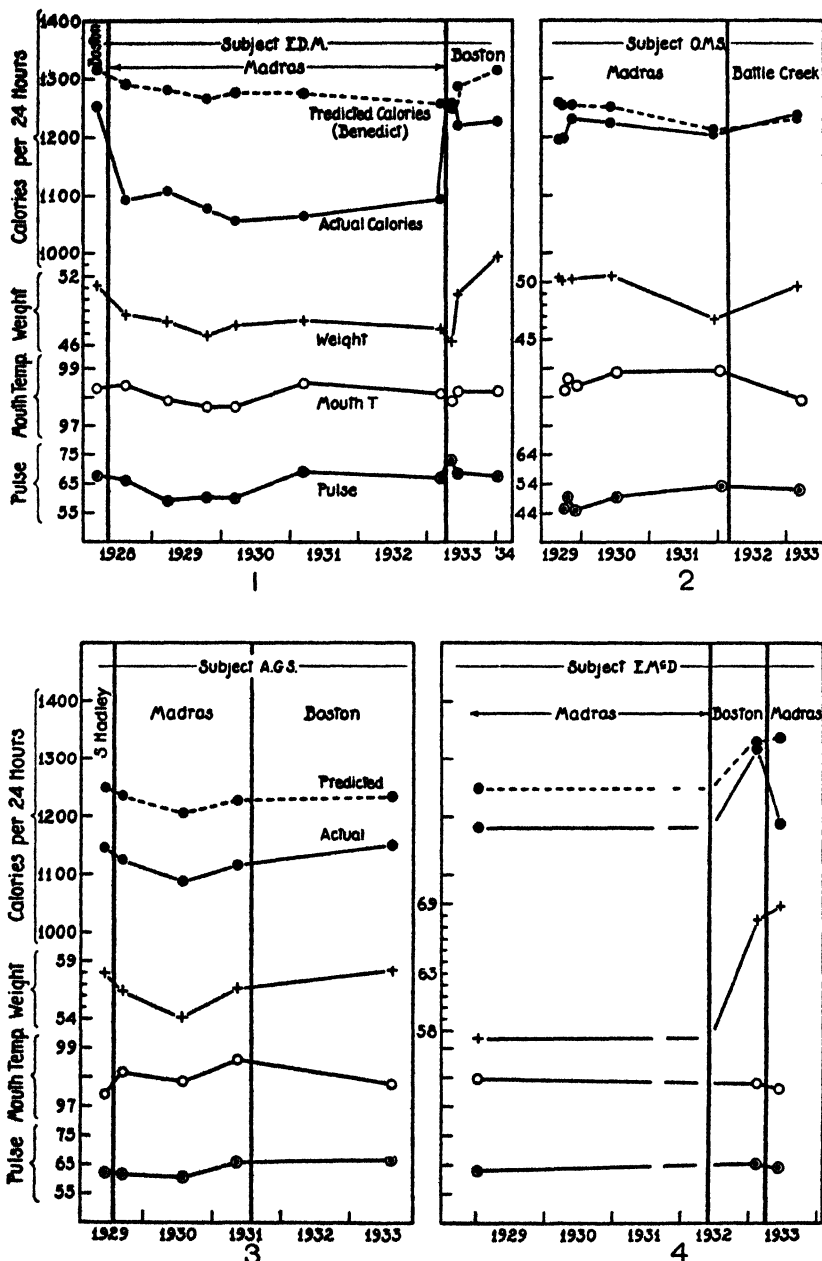
Williams and Benedict ('28) report four Americans measured in Boston and Yucatan. Here again the data are meagre, one preliminary measurement in Boston being followed by one or two in Yucatan and one after return to Boston. All four subjects showed a higher metabolism in Yucatan, varying from the insignificant rise of 1.0 per cent to 9.9 per cent and averaging + 6.4 per cent. It is significant that this change is in the same direction as the metabolism level reported by the same authors for Maya Indians in Yucatan, i.e., the metabolism of the white subjects was higher in Yucatan and the metabolism of the Maya Indians is high.

The most significant study of this kind, even though on only one individual, is that reported by Martin ('30). During a voyage from London to Australia he measured his own basal metabolism with the Douglas bag daily for the 35 days of the voyage, under very constant conditions as regards both food and exercise. Within a few days after encountering hot weather his metabolism began to fall, reaching a minimum on the fifth day of heat and remaining low until cooler weather was brought by the trade winds when it immediately rose to its previous level. The maximum difference between the hot and cool periods was 12 per cent. There is no question that in the case of this individual the tropical climate had a depressing effect on the basal metabolism.

Nine European women studied in Madras and in temperate climates did not show a uniform response to the tropical climate. Three showed a marked decrease in metabolism in the tropics from that in Boston, while a fourth showed a decrease that was definite, but less marked. The measurements on two of these subjects, E.D.M. and E.McD., who differed widely in age and bodily build, are charted in figures 1 and 4. In the most complete series, on E.D.M., the heat production had fallen to a level much below that found in Boston when it was measured for the first time in the tropics after 7 weeks' residence. It remained at this level throughout the 5-year period and when measured 4 days after landing in the U.S.A. had returned to the same level shown in the Boston measurements made 5 years earlier. That the tropical fall in metabolism in this subject was not due to the moderate loss in weight that had occurred in the course of a rough sea voyage is shown by the fact that the very marked rebound in metabolism on her return to a cold climate had taken place while the weight was still at its lowest level. The changes in heat production in the other three of these four subjects were also independent of changes in weight.

In contrast with these four subjects were four others who showed no significant changes in heat production as they moved from one climate to another and a fifth whose measurements showed considerable variation apparently not related to climate. The results for two of these second four, O.M.S. and A.G.S., are charted in figures 2 and 3. With A.G.S. the small fluctuations in heat production were in the same direction as changes in weight, but with O.M.S. the high level of heat production in the temperate climate was sustained in the tropics in spite of marked loss in weight.

In none of the subjects was there any indication that, apart from the effect of loss of weight, continued long stay in the tropics caused a progressive lowering of the metabolism, nor in the entire group of thirty-four women was there any relation between length of stay in the tropics and the level of heat production.



Figs. 1 to 4 The effect of change of climate on metabolism, weight, mouth temperature and pulse rate in European women. (Each point is the average of 2 days' measurements, except those on O.M.S. in Battle Creek which are averaged from 4 days.)



Three Indian women studied similarly in two climates all showed a moderate decrease in metabolism in the tropics, averaging 4.8 per cent.

The data for the nine European subjects are summarized in table 3 in which all the measurements made in the tropics are averaged and compared with the averages of measurements in colder climates. The other nine subjects have been divided arbitrarily into two groups. Group I includes those subjects whose fall in oxygen consumption in the tropics was greater than 10 cc. per minute, group II those whose average oxygen consumption in the tropics did not vary by more than plus or minus 5 cc. from the measurements in a temperate climate.

The averages for the series of measurements on Europeans show:

1. That the weight tended to decrease in the tropics in both groups.

2. That although the subject with the highest tropical temperature (E.M.C.) showed an increased pulse rate in the tropics, the other eight showed a decrease in rate which varied from one to seven beats. This decrease was greatest in three of the four women in group I, but was present in four of the five women in group II in spite of a coincident increase in temperature.

3. That there was an average fall in heat production in the tropics of 5.1 per cent and an average rise in body temperature of  $0.2^{\circ}$  F.

4. That the individual variations in heat production ranged from an insignificant increase to a fall of 12.4 per cent and in mouth temperature from a fall of  $0.4^{\circ}$  F. to a rise of  $0.7^{\circ}$ .

5. *That these individual variations fell into two sharply defined groups, one showing a decrease in heat production of from 7 to 12 per cent and no rise in temperature, and the other showing only slight fluctuations in heat production and an increase in temperature of from  $0.2^{\circ}$  to  $0.7^{\circ}$  F.*

The correlation between per cent change in heat production and change of temperature in the tropics was found to be

TABLE 3

*Summary of experiments on effect of change of climate on European women*

SUBJECT	HEIGHT	AGE AT START	METABOLISM PER CENT DEVIATION IN TEMPERATURE CLIMATE		WEIGHT, KILOGRAM			MOUTH TEMPERATURE °F.			PULSE RATE			O <sub>2</sub> CONSUMPTION CC. PER MIN.		HEAT PRODUCTION CALORIES PER 24 HOURS		
			Harris-Benedict	Atwater	Temp.	Trop.	Per cent change	Temp.	Trop.	Change	Temp.	Trop.	Per cent change	Temp.	Trop.	Temp.	Trop.	Per cent change
Group I	cm.																	
E.L.C.	154	25	-4.0	-2.9	44.0	44.8	+1.8	98.2	98.2	±0	64	58	-9.4	172	160	1195	1112	-6.9
E.D.M.	169	30	-4.0	-10.1	50.4	47.7	-5.4	98.1	98.1	±0	70	64	-8.6	178	156	1237	1084	-12.4
G.E.C.	167	44	-4.6	-12.0	72.0	70.0	-2.8	98.5	98.1	-0.4	72	65	-9.7	199	176	1383	1233	-11.6
E.McD.	164	55	-0.6	-9.6	67.6	63.1	-6.7	97.8	97.8	±0	66	64	-3.0	190	171	1320	1188	-10.0
Group II																		
B.S.C.	157	26	-15.4	-16.8	61.4	58.8	-4.2	98.0	98.2	+0.2	62	61	-1.6	171	169	1188	1174	-1.2
K.N.B.	167	37	-11.6	-17.6	58.0	58.4	+0.7	97.6	97.9	+0.3	77	75	-2.6	170	172	1181	1195	+1.2
O.M.S.	159	37	+0.2	-3.9	49.6	49.5	-0.2	97.9	98.6	+0.7	52	49	-5.8	178	174	1237	1209	-2.3
E.M.C.	165	40	-1.8	-7.8	56.6	53.9	-4.8	98.5	98.8	+0.3	74	85	(+14.9) <sup>1</sup>	186	187	1292	1299	+0.5
A.G.S.	154	52	-7.2	-11.7	58.1	55.7	-4.1	97.6	98.2	+0.6	66	63	-4.5	165	160	1146	1112	-3.0
Average																		
All subjects			-5.4	-10.3	57.5	55.8	-3.0	98.0	98.2	+0.2	67	65	-5.0 <sup>1</sup>	179	169	1242	1177	-5.1
Group I			-3.3	-8.7	58.5	56.4	-3.3	98.2	98.05	-0.15	68	63	-7.7	185	166	1284	1152	-10.2
Group II			-7.2	-11.6	56.7	55.3	-2.5	97.9	98.3	+0.4	66	67	-3.6 <sup>1</sup>	174	172	1209	1198	-1.0

<sup>1</sup> Including E.M.C. the average change in pulse rate for all subjects is -3.4 per cent and for group II +0.1 per cent.

$0.717 \pm 0.109$ , that between change of metabolism and change of pulse  $0.627 \pm 0.136$ . A very interesting physiological adaptation to the tropics is suggested by the first high correlation, namely, that those individuals who are able to diminish their heat production in the tropics are thereby able to avoid a rise of temperature. They may even overdo the adaptation, as is suggested by the temperature curve of E.D.M. (fig. 1), and lower their heat production to such an extent that the body temperature also is lowered. On the other hand, in those individuals who do not respond to the tropical climate with a decrease in heat production, the mechanism for heat loss is not adequate to prevent a rise in temperature.

Apart from this relation between change in metabolism and change in temperature and to a less extent between change in metabolism and change in pulse, there was no uniformity within the two groups, the individuals in each group showing a scattering with respect to race, configuration and previous association with the tropics (table 1) as well as to the initial level in temperate climates of metabolism, temperature and pulse.

The highly suggestive correlation discussed above between change in temperature and change in metabolism is based on only nine fairly scattered points and therefore can be considered as only indicative unless confirmed by further data. However, fairly strong confirmation is provided by an analysis of the relations between individuals in the larger group of thirty-four women measured in the tropics. In this group there was the significant correlation of  $0.530 \pm 0.083$  between mouth temperature and heat production per square meter—a fact which gives considerable support to the adaptation suggested by the group of nine. On the other hand, the less strongly indicated correlation between change in pulse rate and change in metabolism is not supported by the larger group who showed no significant correlation between pulse and metabolism.

Until a long continued study of many individuals has been made, it is possible only to speculate as to whether individuals

showing both types of response to the tropics indicated above may exist in large numbers, or whether an individual who at one time responds in one way may at another time respond differently. It is probable that as the study is continued the division will be found to be less sharp. The fact that the average temperature of the group of thirty-four women in Madras is as high as that of the women in group II suggests that individuals of the second type, whose metabolism remains unchanged, but whose temperature is raised, may predominate in this group.

#### FACTORS OTHER THAN CLIMATE

In discussions of white races living in the tropics one encounters repeatedly the impression that such tropical residents lead a rather indolent existence and that this lack of physical activity accounts for any physiological changes that may occur. In the case of the women studied in Madras this traditional inactivity is certainly a fiction. Of the thirty-four women whose measurements are reported here, thirty were educational or medical missionaries leading exceedingly busy lives and the remaining four were also engaged in exacting activities. In a study of a group composed largely of missionaries one would be inclined to suspect fatigue rather than inactivity as a factor likely to affect metabolism. That this was not a factor is shown from a study of the data presented with relation to the time since last arrival in India. There is no correlation between the time of residence in India and the metabolism either in the group as a whole or in individuals studied through progressive stay in Madras.

The same differences in recreational activity that would be found in a similar group in a temperate environment were apparent here. Individuals of athletic habit at home continue the athletic habit in India, in the forms of tennis, swimming, bicycling, riding, walking, and, in the holidays, climbing.

With regard to diet also, there is a much less radical change than is frequently supposed. While many Europeans have rice and curry frequently, their diet is predominantly Euro-

pean and, as far as I have observed, does not show any indication of decrease in quantity. At the Women's Christian College, where sixteen of the subjects were resident, breakfast, tiffin and dinner were as hearty as the three corresponding meals of the same class of women in America and, in addition, there was a light early morning choti (tea, toast and fruit) and a substantial tea.

While it does not seem probable that there are changes in activity and diet sufficient to account for the changes in metabolism that occur in some individuals, these factors cannot be dismissed with certainty until they have been carefully investigated.

With regard to the influence of factors other than climate in the case of the three Indian subjects measured in temperate climates, it may be stated that all three were engaged in scientific work both in Madras and abroad, that one, a keen botanist, resumed her botanical expeditions on her return to Madras and played tennis. During her first year in Ann Arbor she had lived on a predominantly American diet, but during her second year there returned entirely to her normal Indian vegetarian diet. The second, an excellent tennis player, continued her vigorous game when she returned from Toronto to Madras, and by preference lived on the same diet as her European colleagues. In the case of these two, then, of their own accord the factors of diet and activity were controlled and only climate varied. The third subject was a physically inactive person in Madras and was not given to exercise in London where she was living in a student hostel.

#### CONCLUSION

Returning from the study of these individuals undergoing change of climate to the question of the importance of the climatic factor in the average metabolism level of south Indian women, the following tentative conclusions may be drawn: that since the small group of three Indians showed an average rise of metabolism in colder climates of 4.8 per cent and the group of nine Europeans showed an average decrease in the

tropics of 5.1 per cent, probably at least 5 per cent of the low metabolic level of Indian women may be attributed to the effect of tropical climate. If the 5 per cent correction of standards for women be applied in addition to the correction for climate there still remains to be explained by factors other than climate a difference between the average metabolism of Indian women and western women of approximately 7 per cent.

#### SUMMARY

Measurements on thirty-four European women resident in the city of Madras, which has a mean annual temperature of 82.8° F. and a relative humidity of 72 per cent, showed an average metabolism 7.9, 6.3 and 12.5 per cent below the Harris-Benedict, Dreyer and Aub-DuBois standards, respectively. By the same standards this was 9.0, 9.9 and 4.7 per cent above the average metabolism of Indian women in Madras. The mouth temperature was slightly higher than reported in Western countries and the pulse and blood pressure as low or slightly lower. These three measurements do not differ significantly from those on Indians. The European vital capacity was normal and very much higher than that of Indian women.

Nine European women studied in both temperate and tropical climates showed two types of response to the tropics. One group showed a marked decrease in metabolism, a fairly marked fall in pulse rate and no rise in mouth temperature. The other group showed no change in metabolism, a slight fall in pulse rate and a rise in temperature of from 0.2° F. to 0.7°. The high correlation found in this small group of subjects between change in metabolism and change in temperature is supported by a high correlation between temperature and metabolism in the series of thirty-four women measured in Madras. The average decrease in metabolism of the group of nine moving to the tropics was 5.1 per cent. Three Indian women measured in two climates showed an increase in metabolism of 4.8 per cent in cold climates. These data sug-

gest that approximately 5 per cent of the low metabolism previously reported for Indian women may be attributed to the effect of tropical climate.

#### LITERATURE CITED

- AUB, J. C., AND E. F. DUBOIS 1917 The basal metabolism of old men. *Arch. Int. Med.*, vol. 19, p. 823.
- BENEDICT, F. G. 1928 Basal metabolism data on normal men and women (series II) with some considerations on the use of prediction standards. *Am. J. Physiol.*, vol. 85, p. 607.
- 1930 A helmet for use in clinical studies of gaseous metabolism. *New Eng. J. Med.*, vol. 203, p. 150.
- DREYER, GEORGES 1920 The normal basal metabolism in man, and its relation to the size of the body and age, expressed in simple formulae, *Lancet*, vol. 199, p. 289.
- GRIFFITH, FRED R., G. W. PUCHER, K. A. BROWNELL, J. D. KLEIN, AND M. E. CARMER 1929 Studies in human physiology. 1. The metabolism and body temperature (oral) under basal conditions. *Am. J. Physiol.*, vol. 87, p. 602.
- GUSTAFSON, FLORENCE L., AND F. G. BENEDICT 1928 The seasonal variation in basal metabolism. *Am. J. Physiol.*, vol. 86, p. 43.
- HARRIS, J. A., AND F. G. BENEDICT 1919 A biometric study of basal metabolism in man. *Carnegie Inst. Washington*, publication no. 279.
- HARVEY, O. L., AND H. E. CROCKETT 1932 Individual differences in temperature changes in women during the course of the menstrual cycle. *Human Biology*, vol. 4, p. 453.
- KNIPPING, HUGO WILHELM 1923 a Ein Beitrage zur Tropenphysiologie. Untersuchungen des Gaswechsels, der Blutregeneration, des Blutdruckes, der Atemmechanik, des Wärmehaushaltes in den Tropen und der meteorologischen Faktoren des Tropenklimas. *Zeitschr. Biol.*, Bd. 78, S. 259.
- 1923 b Über die Wärmebalanz des Tropenbewohners. *Arch. Schiffs- und Tropenhygiene*, Bd. 27, S. 169.
- MARTIN, C. J. 1930 Thermal adjustment of man and animals to external conditions. *Lancet*, vol. 219, p. 617.
- MASON, ELEANOR D. 1932 Standards for predicting the normal vital capacity of the lungs in south Indian women from height, weight and surface area. *Indian J. Med. Research*, vol. 20, p. 117.
- MASON, ELEANOR D., AND F. G. BENEDICT 1931 The basal metabolism of south Indian women. *Indian J. Med. Research*, vol. 19, p. 75.
- , 1934 The effect of sleep on human basal metabolism, with special reference to south Indian women. *Am. J. Physiol.*, vol. 108, p. 377.
- VON PIRQUET, CLEMENS 1919 *System der Ernährung*. Berlin.
- 1922 *An Outline of the Pirquet System of Nutrition*. Philadelphia and London.
- RADSMÅ, W. 1931 Metabolism of the European during rest in the tropics. *Arch. Néerland. de Physiol. de l'Homme et des Animaux*, vol. 16, part 1, p. 91.

- TURNER, ABBY H. 1927 a The circulatory minute volumes of healthy young women in reclining, sitting and standing positions. *Am. J. Physiol.*, vol. 80, p. 601.
- 1927 b The adjustment of heart rate and arterial pressure in healthy young women during prolonged standing. *Am. J. Physiol.*, vol. 81, p. 197.
- 1930 Vital capacity in college women. I. Standards for normal vital capacity in college women. *Arch. Int. Med.*, vol. 46, p. 930.
- WILLIAMS, G. D., AND F. G. BENEDICT 1928 The basal metabolism of Mayas in Yucatan. *Am. J. Physiol.*, vol. 85, p. 634.
- YAGLOU, C. P., A. D. BRANDT, AND L. C. BENJAMIN 1933 Observations on a group of subjects before, during and after exposure to ionized air. *J. Indust. Hyg.*, vol. 15, p. 341.





# THE ASSIMILATION OF PHOSPHORUS FROM DICALCIUM PHOSPHATE, C.P., TRICALCIUM PHOSPHATE, C.P., BONE DICALCIUM PHOSPHATE AND COOKED BONEMEAL

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In a review, briefly published ('32) by one of the writers of experiments dealing with the relative value of different forms of calcium and phosphorus as mineral supplements for bone formation, the preponderance of the evidence failed to show any differences among the various forms. However, there were certain possible upsetting factors present in most of these experiments which suggested that the negative data should not be accepted as final. In very few of the comparisons could it be said with certainty that the level of intake was sufficiently low to bring out any difference in efficiency which might exist. In many of the studies involving sources of both calcium and phosphorus, only one of these elements was held constant, with a resulting variable calcium-phosphorus ratio. It seemed worth while therefore, to study this problem further, with special attention to the elimination of these variable factors. The present paper describes such studies with various calcium phosphates.

In planning these studies it became apparent that it is impossible to make a critical comparison of the assimilation of both calcium and phosphorus in the same experiment. For example, if dicalcium phosphate and tricalcium phosphate are introduced into diets 1 and 2, respectively, at the same calcium level, diet 1 will have the more phosphorus and the diets will

differ as regards calcium-phosphorus ratio. This is not a satisfactory situation for the study of the assimilation of either element. The phosphorus levels can be equalized by adding phosphate to diet 2. This provides a suitable set-up for comparing the calcium phosphates as regards calcium assimilation, but not phosphorus, since another source of the latter has been added. Similarly, a satisfactory comparison of the phosphorus of the two sources can be provided for by introducing them at the same phosphorus level and adding more calcium to the dicalcium-phosphate diet. Thus it is possible to study only one of the elements at a time. The

TABLE 1  
*Analyses of mineral supplements*

	CALCIUM	PHOSPHORUS
	<i>per cent</i>	<i>per cent</i>
Dicalcium phosphate, c.p.	24.36	20.49
Tricalcium phosphate, c.p.	36.80	19.00
Bone dicalcium phosphate <sup>a</sup>	23.28	18.67
Cooked bonemeal <sup>b</sup>	31.50	14.84

<sup>a</sup> Obtained from United Chemical and Organic Products Company, Chicago.

experiments here reported deal with the assimilation of phosphorus from the supplements shown in table 1. The bone dicalcium phosphate and the cooked bonemeal were products prepared for human use, being by-products of the manufacture of edible gelatin. Three separate experiments have been carried out.

#### *Comparison of dicalcium phosphate, c.p., and cooked bonemeal*

For this comparison a basal diet low in both calcium and phosphorus was selected as follows:

Butter	10
Patent flour	80
Lactalbumin	7
Yeast concentrate, Harris	1
Salt mixture	2

The salt mixture was a modification of the Osborne and Mendel product, free from calcium and phosphorus. This basal diet contained 0.05 per cent of calcium and 0.14 per cent of phosphorus. Realizing the importance of making the comparison with the element under study at a minimum level, as has been mentioned, and being uncertain as to what that level should be, it was decided to use three different levels in this first comparison. It was decided to maintain a calcium-phosphorus ratio of approximately 1.5 to 1 at each level by the addition of the required amount of calcium carbonate. The first six diets shown in table 2 were accordingly made up by combining appropriate amounts of the supplements with the basal diet. It is seen from the analytical data in the last two lines of the table that diets 1 and 2 provided a comparison at a level of 0.25 per cent, diets 3 and 4 at a level of 0.36 per cent and diets 5 and 6 at a level of 0.47 per cent, the calcium level being approximately 50 per cent higher in each case.

Six pairs of rats, weighing approximately 35 gm. at 23 days of age were selected for each level and fed for 35 days, limiting the food consumption of each pair to the intake of the one consuming the least. At the close of the feeding period the bones of the left hind leg of each rat were excised and analyzed for ash on a dry fat-free basis.

TABLE 2  
*Diets used in growth experiments*

INGREDIENTS	DIETS									
	1	2	3	4	5	6	7	8	9	Control
Basal diet	100	100	100	100	100	100	100	100	100	100
Dicalcium phosphate, c.p.	0.48		1.01		1.52			0.54		
Bonemeal		0.30		1.28		1.97				
Bone dicalcium phosphate							0.59			
Tricalcium phosphate									0.58	
Calcium carbonate	0.58	0.41	0.75	0.31	0.83	0.20	0.58	0.58	0.47	0.53
Calcium, per cent	0.38	0.38	0.55	0.55	0.70	0.70	0.38	0.39	0.38	0.21
Phosphorus, per cent	0.25	0.25	0.36	0.36	0.47	0.47	0.28	0.29	0.30	0.17

*Results.* The bone data from the comparisons at the three phosphorus levels are presented in table 3. At the 0.25 per cent level the figures for percentage of ash favor the dicalcium phosphate in the case of five of the six pairs but, since the odds are only 2 to 1 according to Students' method, the differences have no significance statistically. The weights of bone ash, obtained by multiplying the weight of the dry, fat-free bone by its ash percentage favor the dicalcium phosphate in five out of six of the cases, with odds of 58.5 to 1. At the 0.36 per cent level the data for ash percentage favor the bonemeal in five out of six of the comparisons, but with odds of only 8.6 to 1. The figures for amount of ash favor one supplement in three cases and the other supplement in the other three. At the upper phosphorus level the percentage figures are in favor of the dicalcium phosphate in the case of five out of six of the pairs, with odds of 50.8 to 1. The supplement is also favored as regards bone ash in all cases, with odds of 39 to 1. In summary, the data for percentage ash favor dicalcium phosphate at the lower and upper levels, and the bonemeal at the intermediate level, while the data for amount of ash favor the dicalcium phosphate at all levels. The lack of consistency for the percentage data and the low odds shown for most of the differences rule out any definite conclusion in favor of dicalcium phosphate, from the experiment as a whole.

It is evident from the data that for both supplements the ash percentages were markedly lower at the level of 0.25 per cent phosphorus than at the other two. The average for all the animals fed at the lower level is 50.57 per cent, for the intermediate level, 53.98 per cent and for the upper level, 54.48 per cent. Thus it is clear that at least the level of 0.25 per cent was below that of maximum possible assimilation and that any marked differences in efficiency between the two supplements should have become evident at this level.

TABLE 3  
*Comparison of phosphorus assimilation from dicalcium phosphate, C.P. and cooked bonemeal at different phosphorus levels*

	PHOS- PHORUS LEVEL	PAIR 1		PAIR 2		PAIR 3		PAIR 4		PAIR 5		PAIR 6	
		Dicalcium phosphate, c.p.	Bone phosphate, meal	Dicalcium phosphate, c.p.	Bone phosphate, meal	Dicalcium phosphate, c.p.	Bone phosphate, meal	Dicalcium phosphate, c.p.	Bone phosphate, meal	Dicalcium phosphate, c.p.	Bone phosphate, meal	Dicalcium phosphate, c.p.	Bone phosphate, meal
	<i>per cent</i>												
Ash in dry, fat-free bone, per cent	0.25	48.44	47.43	51.63	50.64	49.77	48.91	50.81	54.23	51.94	48.75	54.13	50.16
Ash in dry, fat-free bone, mg.	0.25	191.0	157.7	190.3	154.7	179.2	166.3	152.3	171.3	161.6	136.1	141.0	124.0
Ash in dry, fat-free bone, per cent	0.36	53.43	55.86	51.44	54.07	53.66	53.82	54.41	55.54	55.26	53.92	52.99	53.33
Ash in dry, fat-free bone, mg.	0.36	212.0	226.7	198.7	205.2	212.0	218.9	189.3	165.9	203.7	182.4	169.1	139.2
Ash in dry, fat-free bone, per cent	0.47	54.34	54.37	54.45	52.01	52.87	52.36	55.77	54.78	56.44	55.87	56.76	54.84
Ash in dry, fat-free bone, mg.	0.47	250.5	227.0	177.5	176.2	198.0	176.7	196.3	192.0	214.6	212.6	246.5	212.0

*Comparison of dicalcium phosphate, c.p., bone dicalcium phosphate and tricalcium phosphate, c.p.*

In view of the data obtained in the first comparison, it was decided to conduct further ones at a phosphorus level of 0.25 per cent, as representing one which was certainly below that of maximum possible phosphorus storage. Diets 7, 8 and 9 listed in table 2 were accordingly made up for use in a second experiment. As is shown in the last line of the table, when the diets were analyzed they were found to be somewhat higher in phosphorus than the planned level. This was due to a larger amount in a new supply of lactalbumin obtained for the basal diet. This larger amount also resulted in a calcium-phosphorus ratio of approximately 1.3 to 1 instead of the 1.5 to 1 used in the first experiment. For comparative purposes, it was desired to ascertain the kind of bone which would be produced at the phosphorus level supplied by the basal ration alone. Thus enough calcium was added to it to provide the same ratio between the elements which was present in the experimental diets. This combination is listed as the control diet in the last column of table 2.

Since three experimental diets were involved, the rats were selected by trios instead of by pairs. Eight trios were used and for a given trio the food intake of the rat consuming the least determined the allowance for all. In addition to these rats distributed among the experimental diets, four comparable animals were placed on the control diet and fed an amount corresponding to the average intake of the other diets. In addition, four comparable animals were killed at the start of the experiment to obtain a measure of the ash content of the bones at this time.

*Results.* The data obtained in this experiment are shown in table 4. In discussing them it is preferable to consider the figures for two supplements at a time. As regards the comparison of dicalcium phosphate, c.p. with tricalcium phosphate, c.p. the data for percentage ash favor the secondary product in five out of the eight cases, but the odds are only 1.5 to 1. A clearer picture is presented by the figures for amount of

TABLE 4

*Comparison of phosphorus assimilation from dicalcium phosphate, C.P., bone dicalcium phosphate and tricalcium phosphate, C.P.*

	DICALCIUM PHOS- PHATE C.P.	TRICALCIUM PHOS- PHATE, C.P.	BONE DICALCIUM PHOS- PHATE	DICALCIUM PHOS- PHATE C.P.	TRICALCIUM PHOS- PHATE, C.P.	BONE DICALCIUM PHOS- PHATE	DICALCIUM PHOS- PHATE C.P.	TRICALCIUM PHOS- PHATE, C.P.	DICALCIUM PHOS- PHATE C.P.	BONE DICALCIUM PHOS- PHATE	DICALCIUM PHOS- PHATE C.P.	TRICALCIUM PHOS- PHATE, C.P.	BONE DICALCIUM PHOS- PHATE
	Trio 1			Trio 2			Trio 3			Trio 4			
Ash in dry, fat- free bone, per cent	53.46	54.99	54.28	53.68	50.15	52.09	51.10	53.34	53.46	51.58	55.79	55.06	
Ash in dry, fat- free bone, mg.	277.5	235.1	256.9	209.1	199.2	223.7	195.9	205.0	200.1	261.9	236.9	277.3	
	Trio 5			Trio 6			Trio 7			Trio 8			
Ash in dry, fat- free bone, per cent	53.15	53.91	54.83	57.82	55.35	57.29	56.37	54.73	57.72	55.78	53.43	55.34	
Ash in dry, fat- free bone, mg.	227.3	226.8	227.2	236.0	220.9	268.5	231.1	209.9	234.3	227.0	205.5	242.6	



ash, since they favor the secondary product in all but one comparison, with odds of 70 to 1. Comparing the data from the bone dicalcium phosphate with those from the tricalcium phosphate, it is found that the bone product resulted in a higher ash percentage in six out of eight cases, with odds of 40 to 1. Similarly, the figures for amounts of ash are larger for the bone product in seven out of the eight comparisons, the odds being 26 to 1. These results which generally tend to favor the dicalcium phosphate, c.p. and the bone product over the tricalcium phosphate were obtained despite the slightly higher percentage of phosphorus in the diet containing the tertiary phosphate, shown in table 2. As regards a comparison of the data for dicalcium phosphate, c.p. and the bone product the percentage data favor the latter in five out of eight cases, but with odds of only 9.4 to 1. The differences in ash content are in the same direction in six out of eight comparisons, but again the odds are very small. Thus none of the data significantly favor one dicalcium phosphate over the other.

Summarizing the data in table 4, no evidence is presented for any difference between the two dicalcium phosphates. However, both of them produced, on the average, higher ash percentages and larger amounts of ash than resulted with the tricalcium phosphate. But the differences are rather small and not accompanied by adequate odds in all cases. Thus, while the data suggest a more efficient utilization of the phosphorus in dicalcium phosphate than in the tertiary product, in agreement with the majority of the data in the first experiment; it is unsafe to consider this entirely proved. Certainly, no large differences exist, and in practice where it may be expected that any supplement used would be added in amounts above the minimum level any small difference in efficiency would become of no importance.

It is evident from the data in table 2 that the supplements furnished only about one-half of the phosphorus intake, and that the results measure the combined effect of the minerals in the basal diet and in the supplements. However, the data

obtained for the rats fed the control diet showed that it produced very poor bone by itself. The bones of the check animals killed at the start contained, on the average, 43.4 per cent of ash. The animals carried through on the control diet, while they made approximately 85 per cent of the gains made where the supplements were used, developed a very poor bone. The bones averaged only 44.1 per cent of ash, compared to an average value of over 54 per cent for the bones of the rats fed the supplements. When these figures are compared with the value for the check animals killed at the start the

TABLE 5  
*Diets used in growth and lactation experiment*

INGREDIENT	DIETS		
	Control (10)	Bone dicalcium phosphate (11)	Bone meal (12)
Butter	10	10	10
Patent flour	80	80	80
Yeast concentrate, Harris	1	1	1
Salt mixture	2	2	2
Lactalbumin	7	7	7
Bone dicalcium phosphate		0.51	
Cooked bonemeal			0.61
Calcium carbonate	0.53	0.65	0.74
Calcium, per cent	0.25	0.43	0.43
Phosphorus, per cent	0.17	0.28	0.28

large influence of the added supplements is evident. Clear proof is furnished that the phosphorus of all the supplements was utilized for bone development.

*A comparison of bone dicalcium phosphate and cooked bonemeal for growth and lactation*

In this experiment the supplements were studied with female rats carried from weaning through their first lactation. The diets used are shown in table 5. It is noted that the control diet and the phosphorus levels were the same as in the second experiment. Ten rats were placed on each diet

at weaning and fed ad libitum. At 4 months of age they were mated with males of proved fertility and at parturition the young of each litter were reduced to six. Eighteen days later, mother and young were killed, after taking a blood sample from the heart of the mother for the determination of inorganic phosphorus. The left femur of the mother was removed for ash analysis. The object of this procedure was to measure the value of the supplements, not only in terms of growth of mother and young, but also as to the state of the phosphorus nutrition of the mother at the close of the lactation period.

All of the animals placed on the experiment lived, grew at a nearly normal rate and were normal in appearance when bred at 4 months of age. As was anticipated, a few of them failed to produce young. Any animal which failed to become pregnant within 2 months after being placed with the male was killed, since it was considered that even though such an animal should prove fertile later the results would be of little value for consideration along with the others of the group. Since only four of the thirty rats were thus killed without producing young, with a distribution of two in one group and one each in the others, as is seen in table 6, this factor does not seriously complicate the interpretation of the results.

*Results.* The data obtained are summarized in table 6. Two sets of averages are presented for each group, one for all of the animals and one for those producing young. The latter is clearly the more useful measure in this study. The gains in weight cover the period from weaning to the time the animals were mated, since after that time the changes in weight were influenced by the onset of pregnancy. These data for gains reveal very good growth in all groups, nearly as good as the average for our stock females. The largest increases were made by the group receiving the dicalcium phosphate. Though suggestive, the differences between the average gains for this group and the other two are not statistically significant. Also it is recognized that without food intake records the possibility of differences in growth

TABLE 6

*Comparison of bone dicalcium phosphate and cooked bonemeal for growth and lactation*

DIET	RAT NO.	GAIN FROM WEAN-ING TO 4 MONTHS	AGE WHEN KILLED	NUMBER OF YOUNG BORN	WEIGHT OF YOUNG REARED	WEIGHT DRY FAT-FREE FEMUR	PER CENT ASH IN DRY FAT-FREE FEMUR	IN-ORGANIC P IN PLASMA
		gm.	days		gm.	gm.		mg./100
Control (10)	1	160	206	0	0	0.3813	64.67	5.7
	2	111	160	6	109	0.2267	54.92	1.4
	3	139	206	0	0	0.3567	66.41	4.9
	4	179	188	7	65	0.3378	61.87	6.0
	5	177	169	5	91	0.2714	57.36	5.8
	6	151	166	6	132	0.2796	58.96	0.6
	17	159	166	4	118	0.2933	56.97	2.8
	18	146	165	6	114	0.2274	56.49	0.7
	19	151	163	9	20	0.3314	61.59	5.4
	20	200	167	7	114	0.2234	57.21	1.8
Average for group		157	176	5.0	76	0.2929	59.64	3.51
Average for fertile rats (8)		159	168	6.2	95	0.2739	58.17	3.06
Bone dicalcium phosphate	7	157	168	5	142	0.3310	62.60	5.0
	8	181	205	7	106	0.3916	64.58	6.5
	9	151	161	6	138	0.3483	63.31	2.8
	10	179	201	8	142	0.3661	64.00	5.0
	11	204	174	7	156	0.3262	63.18	5.7
	26	147	169	6	60	0.4016	65.88	5.3
	27	194	160	4	65	0.4572	67.86	6.3
	28	228	214	0	0	0.4440	66.05	5.5
	29	151	164	6	76	0.3388	64.99	8.5
	30	182	201	8	150	0.3677	62.17	2.5
Average for group		177	182	5.7	103	0.3772	64.46	5.31
Average for fertile rats (9)		172	178	6.3	115	0.3698	64.30	5.29
Cooked bonemeal (12)	12	153	161	5	125	0.3336	62.32	1.9
	13	163	166	5	101	0.3461	63.85	5.2
	14	158	165	4	104	0.3255	63.32	5.6
	15	164	167	6	124	0.2783	63.24	2.6
	16	169	162	6	130	0.3212	62.30	5.0
	21	138	176	6	45	0.3570	64.43	5.0
	22	152	210	0	0	0.4215	65.35	5.0
	23	126	165	5	123	0.3142	64.86	5.5
	24	170	172	7	178	0.3418	63.96	4.4
	25	174	168	9	68	0.4191	66.52	7.0
Average for group		157	171	5.3	100	0.3458	64.01	4.73
Average for fertile rats (9)		157	167	5.9	111	0.3374	63.87	4.70

being due primarily to differences in food intake cannot be ruled out. The striking feature of these growth data is that the very low level of phosphorus in the control diet did not prevent a nearly normal increase in weight. As a result the three groups were nearly alike in weight, as well as being of the same age, when subjected to the test of reproduction and lactation. The data for age when killed are pertinent in connection with the figures for weight and ash content of femur, since the latter values increase with age. It is noted that the group receiving the dicalcium phosphate had a slight advantage in this respect. This was due to the variations in the time elapsing before pregnancy ensued following mating.

The data for reproduction show that two females on the control diet failed to bear young in the time allotted and that the same was true for one animal in each of the other groups. As a whole this represents very good fertility in terms of the records of our colony. For those producing young the average number born is slightly lower for the bonemeal group than for the other two. These averages, as a whole, are close to what may be expected for first litters in our colony. Thus the data cannot be considered to present any evidence that the low phosphorus intake of the control diet interfered with reproduction or that any group was superior to another in this respect.

The weight of young reared constitutes a measure of lactation performance. Since a few of the mothers failed to produce as many as six young, the planned reduction of the litters to this number did not result in entire equality for the lactation study. The differences here were not large, as shown by the following data for the fertile animals:

GROUP	AVERAGE NUMBER AT START OF LACTATION	AVERAGE NUMBER AT END OF LACTATION
Control	5.5	4.7
Dicalcium phosphate	5.7	4.6
Bonemeal	5.3	4.7

These data also indicate the losses which occurred in the course of the lactation and provide a basis for calculating the average individual weight at the close. These lactation data show large individual variations within the groups. For example, in the basal group rat 6 reared all of her six young to a total weight of 132 gm., while rat 19, which gave birth to nine young, reared only one out of six, to a weight of 20 gm. While the highly variable individual data keep the differences from being statistically significant, it is noted that the average values for weight of young reared are distinctly higher for the groups receiving the supplements than for the control, suggesting a beneficial effect from the phosphorus additions.

The more critical measures, however, are the bone data. In this connection it is noted in the table that the average age of the animals when killed was substantially the same for the control and the bonemeal groups, and slightly higher for the other group. The same relations were noted for the weights of the animals when killed. Thus, although the phosphorus additions did not have any marked effect on the growth in weight of the mothers, it is clear from the data for weight of femur that they did markedly influence the growth of bone.

A comparison of the data for diets 10 and 12, for which the average values for weight and age of mothers were nearly identical, shows an increase in femur weight of approximately 23 per cent as a result of the bonemeal addition. The difference is 4.6 times its probable error. For the group on diet 11, in which the average weight of the animals when killed was approximately 15 per cent greater than for the control group, the average femur weight is seen to be 35 per cent higher, the difference being 6.6 times its probable error. While the average value for femur weight is higher for the dicalcium phosphate group than for the group receiving bonemeal, the difference is less than three times its probable error. Further, a higher value is to be expected, since the animals receiving the dicalcium phosphate were heavier and older when killed. Thus no reliable evidence in its favor is here furnished.

The data for ash percentage show clearly that the supplements resulted in a bone of better quality, as well as the larger growth previously discussed. The difference between the average values for the control group and for the dicalcium phosphate group is over eight times its probable error. The difference favoring the bonemeal group over the control has an even higher significance attached to it. It is noted that the values for the two groups receiving the supplements are substantially alike. Experiments with various species have shown that during lactation the calcium and phosphorus in the milk may be in part supplied by a withdrawal of these elements from the bones. It is of especial significance, therefore, that although the groups receiving the supplements presumably secreted more milk and thus more of the minerals, as indicated by the greater weights of young weaned, their bones remained markedly higher in ash at the end.

The data for inorganic phosphorus in the plasma, while highly variable, serve to confirm the conclusions thus far drawn as to the better state of mineral nutrition caused by the addition of the supplements. The difference between the values for the two supplements has no statistical significance.

The foregoing discussion of the bone and blood data in table 6 has been made on the basis of the average values for the animals rearing young, since they represent the more critical measures. It is interesting to compare the data for the rats not producing young in the control group with the average value for the others of the group. For these two rats the figures for weight of bone, ash content and blood phosphorus are all higher than the corresponding average values for the others. This would seem to reveal the draft on the bones which especially results when an animal in a poor state of calcium and phosphorus nutrition is subjected to the strain of lactation. No such differences are shown in the other two groups, but comparison here is of little value, because only one animal in each group did not lactate.

As a whole, the data in table 6 show that, while the low mineral content of the basal diet did not interfere noticeably with growth in weight and thus that no superiority for either supplement was revealed in this respect, their addition did markedly improve bone development. Better bones was undoubtedly produced during the growing period, and this bone presumably remained more nearly intact during pregnancy and lactation than did the bone of the animals on the mineral-deficient, basal diet. At any rate, the animals receiving the supplements were in a much better state of calcium and phosphorus nutrition at the close of the lactation. The usefulness of both bonemeal and dicalcium phosphate as supplements to a mineral-deficient diet is thus clearly shown.

#### SUMMARY AND CONCLUSIONS

Three experiments have been carried out with rats in which the phosphorus intake was kept at a minimum level, and in which the calcium-phosphorus ratio was held constant in the diets under comparison. In the first experiment dicalcium phosphate, c.p., and cooked bonemeal were compared by the paired feeding method at three levels of phosphorus intake, using growth and bone development as the criteria. In the second experiment a similar procedure was used to compare dicalcium phosphate, c.p., bone dicalcium phosphate and tricalcium phosphate, c.p. In the third experiment bone dicalcium phosphate and cooked bonemeal were studied with female rats carried from weaning through their first lactation. In all of these experiments the usefulness of the supplements was shown by the data for ash content of the bones. In the last experiment, although the rats receiving the supplements reared a greater weight of young, their bones remained much higher in both percentage and amount of ash than did those of a control group receiving no supplement. This evidence of a better state of calcium and phosphorus nutrition was supported by data showing a higher level of inorganic phosphorus in the blood. Throughout these three experiments such differences as were shown between the supplements



under comparison generally favored a secondary phosphate over a tertiary product, but we are unwilling to consider this combined evidence as certain proof, because of the small differences involved and the variability of the individual data. It seems very unlikely that any differences which may actually exist are large enough to be of appreciable importance in the selection and use of mineral supplements in practice.

#### LITERATURE CITED

- MAYNARD, L. A. 1932 The value of different calcium supplements in animal feeding. Proc. Am. Soc. An. Prod., pp. 303-308.

# THE SEASONAL VARIATION IN THE ANTIRACHITIC EFFECTIVENESS OF SUNSHINE <sup>1,2</sup>

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The fact that sunshine has distinct antirachitic properties has been so definitely established that citation of further proof is no longer necessary. The results of numerous investigators, however, show that the antirachitic effectiveness of sunshine varies with altitude, latitude, time of day, cloudiness and the amount of dust and moisture in the atmosphere. It has been shown also that there is a seasonal variation in the antirachitic effectiveness of sunshine coincident with the change in the inclination of the sun and with increased cloudiness in winter and increased skyshine in summer (Hess, '25; Hill, '27-'28; Mayer, '26; Russell, et al., '32; Tisdall and Brown, '27 a, b and '29). Since the antirachitic factor is required for reproduction, growth, maintenance of health and prevention of rickets, the character of the climate in any locality is of importance in determining to what extent sunshine can be relied upon as an antirachitic agent.

For this reason it was felt desirable to study the variation in the amount of antirachitically effective ultraviolet rays in sunshine at Ithaca, New York, and thus in the Great Lakes storm-belt area which possesses a climate closely comparable to Ithaca, since the amount of sunshine in this region is much

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less, particularly in the winter season, than in the remainder of the United States, except in the extreme Northwest.

The climatic and atmospheric conditions prevailing at Ithaca are as follows: latitude 42.5 degrees N., elevation 928 feet, average hours of sunshine 44 per cent, mean relative humidity 78 per cent, mean annual rainfall 32.9 inches, considerable snow and little dust or smoke.

When this study was started, little work had been done to determine the actual seasonal variation in the antirachitic effectiveness of sunshine in the different parts of the country, and no work had been reported wherein chicks were used as the experimental animal. Tisdall and Brown ('27 b), using the albino rat, showed that the antirachitic effect of sunshine at Toronto, Canada, in April and May is approximately eight times that in December, January and February. The rat, however, is not very sensitive to a lack of the antirachitic factor. Unless the diet contains a sub-optimum level of phosphorus and the calcium-phosphorus ratio is unduly widened, typical rickets cannot be developed in the rat. Chicks, on the other hand, are extremely sensitive. Even in the presence of a large excess of calcium and phosphorus, rickets, although somewhat delayed, invariably develops and in a severe form.

On account of this and also for certain practical considerations chicks were used as the experimental animal in the investigations reported here. The results obtained serve to check the results of Tisdall and Brown and show the relative value of the chick and the rat in rachitic studies.

The work of Bethke, Kennard and Kik ('25) and Heuser and Norris ('28-'29) shows that relatively small amounts of sunshine are needed to satisfy the antirachitic requirements of the growing chick. Furthermore, Russell and Massengale ('27-'28) and Scott, Hart and Halpin ('29-'30) have reported that sufficient antirachitically effective ultraviolet rays can be obtained from winter sunshine, at least in the regions studied, to prevent the development of rickets in chicks. None of these investigations, however, demonstrates the minimum

requirement of the chick during the different seasons of the year.

#### EXPERIMENTAL PROCEDURE

Because of the inclemency of the weather at Ithaca, it is not possible to expose chicks to direct sunshine during the late fall, winter and early spring. It was, therefore, necessary to make exposures behind a glass which transmits a known percentage of the antirachitic portion of the ultraviolet rays of sunshine. Corex-G980A was used, since this glass trans-

TABLE 1  
*Length of daily exposure of chicks to sunshine for each period*

TREATMENT	ACTUAL DAILY EXPOSURES					
	Period 1 Dec. 22- Mar. 15 1928	Period 2 Mar. 22- June 14, 1928	Period 3 June 21- Sept. 13, 1928	Period 4 Nov. 9- Feb. 1, 1929	Period 5 Feb. 8- May 2, 1929	Period 6 May 10- Aug. 2, 1929
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i> <sup>2</sup>
Window glass	240	40	20	40	20	10
Corex-A glass	30	5	2½	5	2½	1½
Corex-A glass	60	10	5	10	5	2½
Corex-A glass	120	20	10	20	10	5
Corex-A glass	240	40	20	40	20	10
Negative control	....	0	0	0	0	0
Light control	....	30 <sup>1</sup>	30 <sup>2</sup>	30 <sup>1</sup>	30 <sup>1</sup>	10 <sup>2</sup>

<sup>1</sup> Carbon arc

<sup>2</sup> Direct sunshine (not exposed on rainy days)

<sup>3</sup> Exposures were made on alternate days for twice the time indicated, since it was impractical to attempt as short an exposure as 1½ minutes.

mits practically all of the effective ultraviolet rays (Coblentz, '28) and is not subject to any appreciable amount of solari-  
zation (Coblentz, '29).

Four exposing pens glazed with Corex-A and a control pen glazed with common window glass were used each season. In periods 2, 3, 4, 5 and 6 another control pen, receiving irradiation either from a twin carbon-arc lamp with 13 mm. 'therapeutic B' carbons at a distance of 36 inches from the floor or from direct sunshine, was included in the experimental work. Table 1 shows the exposures for the different periods.

All exposures were made at such a time that each period centered at high noon, the time when the sun is perpendicular to the meridian at Ithaca, 76.5°W.

The exposing pens were built on the south side of the brooding laboratory and were 4 feet square. The Corex glass covering the exposing pens was placed at an angle of 47.5° from the horizontal so that the sun's rays would be perpendicular to them at the spring and fall equinoxes. Approximately 13 square feet of glass were available for the transmission of light. The exposing pens were so constructed that the entering light was limited to that coming through the windows. The only other light received by the chicks was from 60 watt Mazda lamps. A standard 14-hour day was used in all seasons.

It was the original intention to arrange the experimental periods so that the summer periods would center at the summer solstice, the winter periods at the winter solstice and the spring periods at the spring equinox. However, it was impossible to start the first winter period until December 22nd. Consequently, the first three periods respectively follow rather than center at these dates. Since there was only 1 week between each of the 12-week periods, the fall period was omitted in 1928 in order that the winter period could start 6 weeks before the winter solstice. Limited time prevented the study of the fall period in 1929.

In all the periods, except the sixth, the chicks behind window glass and Corex-A were exposed daily regardless of the weather. The chicks in the sunshine control pens in periods 3 and 6 were not exposed on rainy days. In the sixth period it was thought impractical to attempt so short an exposure as 1.25 minutes. To make all pens comparable, the Corex-A, window glass and sunshine control pens were exposed on alternate days for twice the time given in tables 1 and 6.

Thirty day-old White Leghorn chicks of uniform breeding were put in each lot in all experiments. Each chick was weighed weekly and the weekly food consumption of each lot was recorded. At the time of weighing observations were

made of the rachitic condition of each chick as determined by the characteristic lameness. These were recorded by degrees of 1 to 4, 1 denoting slight lameness, 2 moderate lameness, 3 severe lameness and 4 extremely severe lameness. The weekly per cent degree of rachitic lameness was found by dividing the summation of the degrees by four times the number of birds present (four representing the theoretical maximum) and multiplying the result by 100. This measure was helpful in comparing the severity of rickets particularly in lots in which all the chicks were rachitic. Average weights were weighted for the influence of sex on growth.

TABLE 2  
*Composition and analyses of rations used*

INGREDIENTS	PERIOD 1 (WINTER)	PERIOD 2 (SPRING)	PERIOD 3 (SUMMER)	PERIOD 4 (WINTER)	PERIOD 5 (SPRING)	PERIOD 6 (SUMMER)
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Ground yellow corn	52.5	52.5	57.0	57.5	57.5	57.5
Flour wheat middlings	20.0	20.0	20.0	20.0	20.0	20.0
Meat scrap (55 per cent protein)	10.0	10.0	....	....	....	....
Dried buttermilk	10.0	10.0	20.0	20.0	20.0	20.0
Alfalfa leaf meal	5.0	5.0	....	....	....	....
Steamed bone meal	2.0	2.0	2.5	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Per cent protein	17.3	17.3	14.9	14.9	14.6	15.0
Per cent calcium	1.968	2.210	1.185	0.901	0.897	0.955
Per cent phosphorus	1.186	1.316	0.985	0.815	0.777	0.774

Table 2 gives the percentage composition and analyses of the diets used in each of the periods. The diets used for the first three periods gave good growth, but did not produce sufficiently severe rickets at an early age and so were changed for the last three periods as indicated.

At the end of the sixth and twelfth weeks of periods 3, 4, 5 and 6 and at the end of the twelfth week of period 2, approximately 5 cc. of blood were taken by heart puncture (Sloan and Wilgus, Jr., '30-'31) from each of eight representative chicks per lot. Then the blood of each lot was pooled and

serum calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method and the serum inorganic phosphorus by the Fiske and Subbarow method as outlined by Hawk and Bergeim ('26).

At the end of the twelfth week of each period the left tibia and femur were taken from each of the eight chicks per lot from which the blood samples were drawn for the determination of bone ash. These bones were dried and freed of fat previous to ashing so that the per cent ash could be expressed on the moisture-free, fat-free basis.

During the last three periods an attempt was made to measure by chemical and physical means the relative amount of antirachitically effective ultraviolet rays present in sunshine. Of the various chemical methods available, the oxalic acid-uranyl sulfate method of Anderson and Robinson ('25) with slight modifications was chosen as being most suitable. Four quartz tubes containing oxalic acid were used. Two tubes were exposed directly to sunshine and the other two were exposed underneath common window glass. It was thought that by subtracting the grams of oxalic acid decomposed beneath the window glass from the grams of oxalic acid decomposed in the direct sunshine the decomposition due to the wave lengths shorter than 3130 Ångstrom units, that is, the waves having the greatest antirachitic effect, could be obtained.

The tubes were exposed on the south side of the analytical laboratory in a box constructed in such a manner as to avoid reflections from the box and also to prevent the freezing of the solutions. The box was mounted on hinges so that the tubes could be adjusted at right angles to the sun's rays during each exposure.

The apparatus used for the physical measurement of the effective ultraviolet rays was the Anderson and Gordon fluorescent ultraviolet photometer ('28) especially adapted for this purpose by Dr. H. P. Gage, of the Corning Glass Works, Corning, New York. This was accomplished by the use of a special uranium glass which fluoresces only when exposed

to ultraviolet rays shorter than 3130 Ångstrom units. The readings of the instrument give the amount of current in milliamperes necessary to bring a small light to the same brilliance as that caused by the fluorescence of the uranium glass from the incident ultraviolet rays of sunshine. These readings are then converted into intensity of antirachitic ultraviolet irradiation by means of a chart prepared by standardizing the instrument against a quartz mercury-vapor arc operated at 77 volts and 3.45 amperes. The intensity of the fluorescence of the uranium glass at 1 meter from the quartz mercury-vapor arc is called a 'Q' unit.

The results, therefore, are only an expression of the relative intensities of ultraviolet rays and not of the actual amount of ultraviolet-ray energy. To get the average intensity for 1 day or part thereof, a series of readings were taken at short intervals. These were made during the time the chicks were being exposed.

### RESULTS

In view of the fact that the first three periods were somewhat of a preliminary nature and that the changes in the rations prevent the making of direct comparisons with the last three periods, it will suffice if only brief mention of the results obtained is made. These are summarized in table 3.

The results of period 1 (winter) indicated that an exposure of 1 hour or more through Corex-A<sup>3</sup> was sufficient to prevent the development of rickets. Although the average weight and bone ash were normal in the lot receiving 30 minutes' exposure, there was one chick recorded as being rachitic. This, considered with the results of studies of other glazing materials of lower transmissibility which accompanied this work, but are not reported here, seemed to indicate that 30 minutes' exposure was very close to the minimum.

The results of period 2 (spring) indicated that an exposure of 10 minutes or more was sufficient to prevent the develop-

<sup>3</sup> Hereafter in speaking of exposures to sunshine, exposures through Corex-A glass will be meant unless otherwise indicated.



ment of rickets. In the lot receiving an exposure of 5 minutes, there was one chick recorded as being rachitic, and the average weight, the grams of bone ash and the percentage of bone ash were slightly below the values for the other Corex and the carbon-arc lots. These results seem to justify the conclusion that an exposure of 5 minutes daily was slightly less than the minimum requirement. When compared with the results of the previous period, a winter to spring ratio of antirachitic effectiveness of sunshine of approximately 1:6 is indicated. A comparison of the sunshine record for these two periods with the 21-year average for these periods indicates that the ratio would have been somewhat narrower in a normal season (table 7).

At the end of period 3 (summer) there were no external symptoms of rickets in any of the lots except the negative controls. However, in the lot receiving the 2.5 minute exposure, the average weight, the grams of bone ash and percentage of bone ash were slightly below normal. Considering these results and those of the studies of other glazing materials previously mentioned, it seems that the exposure of 2.5 minutes daily was slightly less than the minimum protective exposure. Due to the change in ration, this period cannot be compared directly with the two previous periods, but the results indicate roughly a ratio of effectiveness of spring and summer sunshine of approximately 1:2.

A comparison of the increase in hours of sunshine during the periods of exposure for these three periods with the increased antirachitic effectiveness, indicates quite clearly that the increase in effectiveness from winter to spring was due very largely to an increase in intensity, since there was an increase of only 31.6 per cent in the hours of sunshine between 11 A.M. and 1 P.M. The increase in effectiveness from spring to summer was apparently due both to an increase in hours of sunshine as well as to an increased intensity.

A minimum exposure of 5 minutes and a maximum of 40 minutes daily were used in period 4 (winter), since the results of the previous winter period indicated that the length of ex-

TABLE 3

*Summary of results of 12-week-old chicks, periods 1, 2,*

GLAZING MATERIAL	MINUTES' EXPOSURE	PERIOD 1 (WINTER)			PERIOD 2 (SPRING)			PERIOD 3 (SUMMER)		
		Average weight in grams	Grams bone ash	Per cent bone ash	Average weight in grams	Grams bone ash	Per cent bone ash	Average weight in grams	Grams bone ash	Per cent bone ash
Window glass	240,40,20	708	2.16	43.7	530	1.58	41.3	392	1.11	42.7
Corex-A glass	2.5	....	....	....	....	....	....	778	2.20	47.2
Corex-A glass	5	....	....	....	768	2.43	44.6	852	2.48	48.3
Corex-A glass	10	....	....	....	847	2.78	46.3	773	2.38	48.7
Corex-A glass	20	....	....	....	852	2.75	46.7	817	2.63	49.3
Corex-A glass	30	889	2.90	48.9	....	....	....	....	....	....
Corex-A glass	40	....	....	....	810	2.80	45.9	....	....	....
Corex-A glass	60	861	2.78	49.3	....	....	....	....	....	....
Corex-A glass	120	866	2.88	48.0	....	....	....	....	....	....
Corex-A glass	240	914	2.89	47.9	....	....	....	....	....	....
Negative control	0	....	....	....	515	1.54	41.3	402	1.12	41.9
Light control	30	....	....	....	838	2.71	45.8	844	2.66	49.0

TABLE 4

*Summary of results of 12-week-old chicks; period 4, winter; November 9, 1928, to February 1, 1929*

GLAZING MATERIAL	MINUTES' EXPOSURE	AVERAGE WEIGHT IN GRAMS	MILLIGRAMS CA PER 100 CC. BL. SERUM	MILLIGRAMS P PER 100 CC. BL. SERUM	PER CENT RICKETS	PER CENT DEGREE RICKETS	GRAMS BONE ASH	PER CENT BONE ASH
Window glass	40	275.5	8.80	5.90	100.0	98.2	0.766	37.76
Corex-A glass	5	402.4	7.70	6.18	100.0	95.0	1.137	37.62
Corex-A glass	10	516.8	9.92	4.96	90.0	73.8	1.469	38.96
Corex-A glass	20	708.6	9.95	5.68	38.5	23.1	1.975	42.18
Corex-A glass	40	796.9	10.94	5.58	10.7	6.3	2.429	45.53
Negative control	0	263.6	8.54	5.14	100.0	100.0	0.748	36.48
Light control <sup>1</sup>	30	869.6	11.77	5.82	0.0	0.0	2.619	47.35

<sup>1</sup> Carbon arc

posure would have to be reduced to get below the minimum requirement.

A comparison of the results of the 40-minute lot with those of the carbon-arc control (table 4) showed slight but quite evident indications of rickets. The average weight and grams and percentage of bone ash were slightly below the carbon-arc control. Moreover, there were three chicks in this lot with characteristic rachitic lameness, although only one case was severe. The external symptoms of rickets did not appear until the eighth week. These results indicate that, although 40 minutes was not sufficient to entirely prevent rickets, it was close to the minimum requirement.

The number of hours of sunshine for this period was 13.7 per cent greater than the 21-year average. Therefore, for an average season the minimum exposure required would probably be in the neighborhood of 45 minutes.

The results of the fifth period (table 5) indicate that there was considerably more effective ultraviolet rays in this period than in the previous winter period. Although the average weight, serum calcium and percentage of bone ash of the 10-minute lot were normal, there were two chicks at the end of the 12 weeks with slight but definite external symptoms of rickets. One chick died during the eighth week with severe rickets and one of the chicks taken for blood and bone analysis showed slight beading of the ribs. This chick and the one that died were recorded as being moderately rachitic after the fourth week. This shows that 10 minutes' daily exposure was nearly but not completely protective.

The condition of rickets in the 10-minute lot in this period was quite similar to that in the 40-minute lot, period 4. Thus it took approximately four times the exposure in the winter to produce the same results as were obtained with 10 minutes in the spring. This gives an approximate ratio of effectiveness for winter as compared to spring of 1:4, a ratio which is somewhat narrower than was found for the previous winter and spring periods (period 1 and 2).

Periods 1 and 2 are not strictly comparable, respectively, with periods 4 and 5, since the diet used for the latter two periods contained less calcium and phosphorus and the periods of exposure were 6 weeks earlier. However, the ratio of the minimum protective exposures for periods 4 and 5 should be

TABLE 5

*Summary of results of 12-day-old chicks; period 5, spring; February 8 to May 2, 1929*

GLAZING MATERIAL	MINUTES' EXPOSURE	AVERAGE WEIGHT IN GRAMS	MILLIGRAMS Ca PER 100 CC. BL. SERUM	MILLIGRAMS P PER 100 CC. BL. SERUM	PER CENT RICKETS	PER CENT DEGREE RICKETS	GRAMS BONE ASH	PER CENT BONE ASH
Window glass	20	318.7	9.27	6.72	100.0	92.6	0.852	42.85
Corex-A glass	2½	475.4	10.10	6.38	74.1	48.1	1.353	²
Corex-A glass	5	666.9	11.54	6.74	40.7	20.4	1.982	²
Corex-A glass	10	841.0	12.65	6.00	7.4	2.8	2.841	50.39³
Corex-A glass	20	879.4	12.89	5.38	0.0	0.0	2.590	50.68
Negative control	0	308.6	8.83	6.14	100.0	91.2	0.804	40.14
Light control ¹	30	847.1	14.44	6.38	0.0	0.0	2.503	50.48

¹ Carbon arc

² Ash determinations lost

³ Average of five bones; other three lost

TABLE 6

*Summary of results of 12-day-old-chicks; period 6, summer; May 10 to August 2, 1929*

GLAZING MATERIAL	MINUTES' EXPOSURE	AVERAGE WEIGHT IN GRAMS	MILLIGRAMS Ca PER 100 CC. BL. SERUM	MILLIGRAMS P PER 100 CC. BL. SERUM	PER CENT RICKETS	PER CENT DEGREE RICKETS	GRAMS BONE ASH	PER CENT BONE ASH
Window glass	10	360.2	7.89	6.88	100.0	87.5	0.942	36.67
Corex-A glass	1½	615.1	9.78	6.52	62.5	34.4	1.675	41.29
Corex-A glass	2½	626.3	9.86	6.64	34.6	24.0	1.660	41.70
Corex-A glass	5	819.0	10.66	7.12	3.7	0.9	2.383	46.37
Corex-A glass	10	742.0	11.28	7.40	0.0	0.0	2.139	45.56
Negative control	0	390.7	7.74	5.64	100.0	92.1	1.020	35.96
Light control ¹	10	818.1	11.12	6.56	0.0	0.0	2.421	44.03

¹ Direct sunshine

narrower, due to the fact that the amount of sunshine during the hours of 11 A.M. to 1 P.M. in period 5 (spring) was only 10 per cent greater than for period 4 (winter), whereas for period 2 (spring) the amount of sunshine during this time was 31.6 per cent greater than for period 1 (winter). Had there been an increase in the amount of sunshine from winter to spring in periods 4 and 5 comparable to that in periods 1 and 2, it seems probable that the ratio of effectiveness of sunshine from winter to spring, would have been closer to the ratio of 1:6 found for periods 1 and 2. Upon consideration of this and of the 21-year average, it seems probable that the ratio of effectiveness of an average season is slightly narrower than 1:6. Since the increase in the amount of sunshine between the hours of 11 A.M. to 1 P.M. for these periods was not very great, it is evident that the increase in effectiveness of spring sunshine over winter sunshine was due almost entirely to an increase in intensity of ultraviolet rays.

Since an exposure of 2.5 minutes was nearly protective during the previous summer period, the shortest exposure was reduced to 1.25 minutes for period 6. This reduction with the decrease in the calcium and phosphorus content of the diet proved to be sufficient to make the exposure of 1.25 minutes sub-minimal (table 6).

When compared with the sunshine control pen which received 10 minutes' daily exposure to direct sunshine, the chicks which received 5 minutes' daily exposure through Corex-A glass seemed to be nearly normal. The average weight of the latter lot was practically the same as the weight of the sunshine control lot. The percentage of bone ash, however, was slightly lower in the 5-minute Corex lot than that of the sunshine control lot. The serum calcium was also lower in the 5-minute lot than that of the sunshine control lot. These differences do not point conclusively toward a rachitic condition in the 5-minute lot, but, since they are consistent and since there was one chick in this lot which showed slight rachitic lameness at the end of the twelfth week, it seems reasonable to conclude that there was a condition of slight rickets present.

The low average weight of the chicks in the lot receiving 10 minutes' exposure cannot be accounted for. The grams and percentage of bone ash were somewhat lower than in the sunshine control lot, but this is not considered to be conclusive evidence of rickets, since the serum calcium was normal and the lot receiving half the exposure was nearly normal in all respects. There were no external or internal symptoms of rickets.

A comparison of the results of periods 5 and 6 shows that approximately the same degree of rickets prevailed in the 5-minute lot in the summer as in the 10-minute lot in the preceding spring. This shows that about twice the exposure was required in the spring period as for the summer period, making a spring-summer ratio of effectiveness of 1:2.

There was a 55.4 per cent increase in the amount of sunshine in period 6 during the hours of 11 A.M. to 1 P.M. as compared to period 5. This indicates that the increased effectiveness for this period (summer) was due both to an increase in amount of sunshine and to an increase in intensity.

As the condition of rickets in the 40-minute Corex lot in period 4 (winter) was similar to that in the 5-minute Corex lot in period 6 (summer), the exposure required during winter to prevent the development of rickets was about eight times as great as was required for the summer, giving a ratio of effectiveness of 1:8.

In view of the fact that there is some relation between the quantity of sunshine between 11 A.M. and 1 P.M. and the total hours of sunshine, the probable ratio for an average year was calculated. These calculations were based upon the variations in total hours of sunshine of the years 1928 and 1929 from the 21-year average (table 7). The results showed that the calculated winter-spring-summer ratio of effectiveness for an average year, periods 1, 2 and 3, was 1:5.3:9.3 and that of periods 4, 5 and 6 was 1:5.9:8.1. In view of all the variables concerned, this is remarkably close agreement for experimental work of this character. The conclusion seems justified, therefore, that the antirachitic effectiveness of sunshine for the

winter, spring and summer seasons varies in accordance with the ratio 1:5-6:8-9.5.

The increase in effectiveness of sunshine from winter to summer of approximately eight to nine times is in accord with the data of Dorno (Hess, '25), showing that the intensity of the ultraviolet rays of sunshine increased about 8.75 times from January 15th to July 15th. On the other hand, the increase in effectiveness of sunshine from winter to spring was not found to be as great as was found by Tisdall and Brown ('27), who concluded that the antirachitic effect of sunshine in April and May was about eight times as great as in Decem-

TABLE 7

*Total hours and percentage of possible sunshine at Ithaca, New York<sup>1</sup>*

PERIOD	21-YEAR AVERAGE SUNRISE TO SUNSET		1928-29 SUNRISE TO SUNSET		1928-29 11 A.M. TO 1 P.M.		HOURLS 1928-29 ± 21-YEAR AVERAGE
	hours	per cent	hours	per cent	hours	per cent	per cent
1. (Jan.-Mar.)	373.3	38	319.4	33	89.6	49	-14.4
2. (Apr.-June)	679.4	52	658.0	50	117.9	65	- 3.1
3. (July-Sept.)	731.8	58	805.1	63	147.9	80	+10.0
4. (Nov.-Jan.)	230.8	26	262.5	30	76.8	42	+13.7
5. (Feb.-Apr.)	465.2	43	361.0	33	84.5	47	-22.4
6. (May-July)	775.7	57	867.9	63	131.2	71	+11.9

<sup>1</sup> Records of the Ithaca Station of the United Weather Bureau

ber, January and February. Since Toronto, Canada, is also in the Great Lakes storm belt, it is logical to conclude that the slight difference in latitude would not affect the results. It is more probable, therefore, that the difference in results can be ascribed to the difference in the amount of sunshine, since the amount recorded by Tisdall and Brown at Toronto for the 2-hour period, 11 A.M. to 1 P.M., for the months of December, January and most of February, 1926-1927, is less than that recorded for the same period at Ithaca in 1928-1929, while the amount for their 4-week experimental period ending May 9, 1927, at Toronto was slightly greater than that for the same period at Ithaca in 1929.

The possibility that the difference in results was due in part to the use by Tisdall and Brown of the rat as the experimental animal and of a rachitogenic diet in which the quantity of calcium and phosphorus was greatly unbalanced was not revealed by the evidence obtained. Both types of experimental animals on the respective rachitogenic diets used appeared equally sensitive to a lack of the effective ultraviolet rays of sunshine.

These experiments demonstrate quite clearly the small amount of sunshine required even in the winter time, to prevent the occurrence of rickets in chicks. Since only a small part of the United States is in a region of as little average sunshine (44 per cent) as Ithaca, New York (Ward, '25), the conclusion seems justified that sunshine can quite generally be depended on as a source of the antirachitic factor for chicks, if the chicks are exposed directly or behind ultraviolet transmitting materials, the period of exposure depending on the percentage transmission of the material used. In view of the fact that 30 minutes was found to be protective in period 1 during the winter, there are probably three to four times as much antirachitically effective ultraviolet rays available as are necessary for chicks, since it has been shown that in this season the effective ultraviolet rays are at their greatest intensity between the hours of 11 A.M. and 12 M. and that the intensity from 10 A.M. until 11 A.M. and from 12 M. to 1 P.M. is nearly as great (Hess, '25).

It should be remembered that the minimum exposures found were obtained behind glass which transmits a maximum of 92 per cent due to the reflection from the surface (Coblentz, '28). The absolute minimum exposures required without glass, therefore, would be 8 per cent less. This fact is important in estimating minimum exposures under conditions where it would not be necessary to use glass, but it would probably not change the ratio of minimum exposures required in the different seasons.

The relatively high susceptibility of chicks to rickets makes the results of this work very applicable to other types of ani-



mals requiring the antirachitic factor for proper development and maintenance of health and to human beings. Unfortunately, there is no definite information to indicate whether or not humans have a higher requirement for the antirachitic factor than chicks. However, because of the possibility for a greater body-surface exposure, it is probable that where a high-transmitting glazing material is used, sufficient beneficial effect can be obtained in most parts of the United States even in the winter time from the use of the available sunshine as a source of the antirachitic factor even though the period of exposure is limited to a short time during the middle of the day.

TABLE 8

*Amount of sunshine during periods 4, 5, and 6 expressed in milligrams of oxalic acid decomposed and in 'Q' units*

PERIOD	AVERAGE AMOUNT OXALIC ACID DECOMPOSED DAILY				AVERAGE 'Q' UNITS
	Exposure	Direct sunshine	Window-glass filter	Difference	
	min.	mg.	mg.	mg.	
4 (winter)	40	22.19	16.69	2.50	0.618
5 (spring)	10	8.25	7.13	1.12	1.150
6 (summer)	5	6.31	5.44	0.87	3.481

It is probable, moreover, that people working constantly out-of-doors in the winter time have no need for other sources of the antirachitic factor, even though only the face and occasionally the hands are exposed to the available ultraviolet rays. This observation is supported by the fact that in chicks protection against the development of rickets depends upon the exposure of head parts and feet (Maughan, '30), as down and feathers screen out the effective ultraviolet rays the same as the clothing of human beings.

The results of the chemical and physical studies (table 8) did not show the same change in the antirachitic effectiveness of sunshine as did the work with chicks. According to the chemical method, there was an average of 2.5 mg. of oxalic acid decomposed daily during an exposure of 40 minutes by the ultraviolet rays not transmitted by window glass in

period 4 (winter, 1928-1929). In period 5 (spring) the average daily decomposition of oxalic acid was 1.12 mg. during a 10-minute exposure and for period 6 (summer) 0.87 mg. during a 5-minute exposure. If this method had shown the same relationship between the seasons as did the work with chicks, the decomposition of oxalic acid would have been the same for the three periods, since the length of the minimum protective exposures for the chicks during the winter, spring and summer were, respectively, 40, 10 and 5 minutes. The results obtained by this method, therefore, did not show the true relationship of the amount of antirachitically effective ultraviolet energy received by the chicks during periods 4, 5 and 6.

The results obtained by physical measurement approximate the results obtained with chicks more closely. The average daily 'Q' units for the winter, spring and summer periods were, respectively, 0.618, 1.150 and 3.481. As the readings with this instrument are instantaneous, the average 'Q' units would be expected to be greater for the spring than for winter and greater for the summer than for spring. There was a considerable seasonal increase in the readings as is shown by the above values. These give a ratio for the three seasons, winter, spring and summer, of 1:1.86:5.63. This method indicated somewhat more accurately the amount of antirachitically effective ultraviolet rays incident in the three seasons than the other method used, but it does not appear sufficiently reliable to replace biological studies.

#### SUMMARY

1. The minimum daily exposure to sunshine required to prevent the development of rickets in chicks at Ithaca, New York, in 1927-1928 appeared to be approximately 30 minutes during winter, 5 minutes during spring and 2.5 minutes during summer, when the exposures were made behind glass which transmitted practically all the ultraviolet rays of sunshine, except those lost by reflection and when the 12 weeks' experimental period used began at the December and June solstices and the March equinox. With an experimental period of equal

length, but centering rather than beginning at these dates and using a rachitogenic diet possessing a smaller quantity of calcium and phosphorus, the minimum protective exposure in 1928-1929 was found to be approximately 40 minutes during the winter, 10 minutes during the spring and 5 minutes during the summer.

2. When the results obtained in 1927-1928 were calculated in terms of the 21-year average of sunshine, it was found that the winter-spring-summer ratio of effectiveness of an average year was 1:5.3:9.3. The ratio of an average year based upon the 1928-1929 results was found to be 1:5.9:8.1. It appears, therefore, that the antirachitic effectiveness of sunshine for the winter, spring and summer seasons at Ithaca, New York, varies in accordance with the ratio 1:5-6:8-9.5. Since Ithaca, New York, is in one of the two areas in the United States with least amount of sunshine, it is probable that the results obtained in this experimental work are an expression of the minimum antirachitic effectiveness of sunshine in the United States rather than an expression of the average or maximum effect.

3. The increase in antirachitic effectiveness of spring sunshine at Ithaca as compared to winter sunshine was caused largely by an increase in intensity rather than in the amount of sunshine. On the other hand, the increase in effectiveness of summer sunshine as compared to spring sunshine appeared to be due about equally to both increase in intensity and increase in amount of sunshine.

4. The quantity of antirachitically effective ultraviolet rays of sunshine was not accurately shown either by a modification of the oxalic acid-uranyl sulfate method of Anderson and Robinson or by an adaptation of the Anderson and Gordon fluorescent ultraviolet photometer.

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## LITERATURE CITED

- ANDERSON, W. T., AND E. GORDON 1928 A fluorescent ultraviolet photometer. *J. Opt. Soc. Am.*, vol. 16, p. 224.
- ANDERSON, W. T., AND F. W. ROBINSON 1925 The oxalic acid-uranyl sulfate ultraviolet radiometer. *J. Am. Chem. Soc.*, vol. 47, p. 718.
- BETHKE, R. M., D. C. KENNARD AND M. C. KIK 1925 The relation of sunlight and green clover to leg weakness in chicks. *J. Biol. Chem.*, vol. 63, p. 377.
- COBLENTZ, W. W. 1928 Spectral characteristics of light sources and window materials used in therapy. *Trans. Illum. Eng. Soc.*, vol. 23, p. 247.
- COBLENTZ, W. W., AND R. STAIR 1929 Data on ultraviolet solar radiation and the solarization of window materials. *J. Res. U. S. Bureau of Standards*, vol. 3, p. 629.
- HAWK, P. B., AND O. BERGEIM 1926 Practical physiological chemistry. P. Blakiston's Son & Co., Philadelphia.
- HESS, A. F. 1925 The ultraviolet rays of the sun. *J. Am. Med. Assoc.* vol. 84, p. 1033.
- HEUSER, G. F., AND L. C. NORRIS 1928-1929 The effectiveness of mid-summer sunshine and irradiation from a quartz mercury vapor arc in preventing rickets in chicks. *Poul. Sci.*, vol. 8, p. 89.
- HILL, L. 1927-1928 Measurement of the biologically active ultraviolet rays of sunlight. *Proc. Roy. Soc. London*, vol. 102-B, p. 119.
- MAYER, E. 1926 Clinical application of sunlight and artificial radiation. The Williams & Wilkins Co., Baltimore.
- MAUGHAN, G. H., AND J. A. DYE 1930 The amount of ultraviolet radiation necessary to cure rickets. *J. Opt. Soc. Amer.*, vol. 20, p. 279.
- RUSSELL, W. C., C. H. HOWARD AND O. N. MASSENGALE 1932 Four years of daily measurement of the ultraviolet component of sunlight by the acetone methylene blue method. *Am. J. Hyg.*, vol. 16, p. 192.
- RUSSELL, W. C., AND O. N. MASSENGALE 1927-1928 The effect on bone formation of winter sunlight transmitted by a glass substitute. *Poul. Sci.*, vol. 7, p. 85.
- SCOTT, H. T., E. B. HART AND J. G. HALPIN 1929-1930 Winter sunlight, ultraviolet light and glass substitutes in the prevention of rickets in growing chicks. *Poul. Sci.*, vol. 9, p. 65.
- SLOAN, H. J., AND H. S. WILGUS, JR. 1930-1931 Heart probe, a method for obtaining blood samples from chickens. *Poul. Sci.*, vol. 10, p. 10.
- TISDALL, F. F., AND ALAN BROWN 1927 a Seasonal variation of the antirachitic effect of sunshine. *Am. J. Dis. Child.*, vol. 34, p. 721.
- 1927 b Antirachitic effect of skyshine. *Am. J. Dis. Child.*, vol. 34, p. 737.
- 1929 The relation of the altitude of the sun to its antirachitic effect. *J. Am. Med. Assoc.*, vol. 92, p. 860.
- WARD, B. D. 1925 The climates of the United States. Ginn & Co., Boston.



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